Biodegradable thermosensitive copolymer hydrogels for drug delivery

Xian Jun Loh & Jun Li

†National University of Singapore, Division of Bioengineering, Faculty of Engineering, 7 Engineering Drive 1, Singapore 117574, and Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602, Singapore

Biodegradable thermogelling copolymer hydrogels have great applicative potential in areas such as sustained drug release, gene delivery and tissue engineering. These injectable materials can be implanted in the human body with minimal surgical intervention. The thermosensitive copolymers have been incorporated with a variety of biocompatible and biodegradable components such as poly(D,L-lactic acid-co-glycolic acid), poly(L-lactic acid), poly(L-carprolactone), poly([R]-3-hydroxybutyrate), poly(organophosphazene), poly(peptide), poly(propylene fumarate), poly(propylene phosphate), polyacetal and poly(ortho ester). Various formulations consisting of the copolymers and therapeutic agents have been developed and the sustained release of these agents has been demonstrated. This review aims to provide a comprehensive summary of the recent developments in this field of study and highlights the most recent intellectual property and research papers.

Keywords: biodegradable, block copolymer, thermosensitive, hydrogel, drug delivery

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1. Introduction

Drug delivery poses a great challenge to clinicians everywhere. Drugs work at their optimum efficacy when they are maintained at a certain concentration in the blood plasma known as the therapeutic concentration. Below this concentration, the efficacy of the drug decreases and above this concentration, toxicity could result from overdosage. Drugs that are administered by the oral route frequently suffer from losses resulting from first-pass metabolism, reducing the bioavailability of the drug. In cases where the drug suffers from poor oral bioavailability of the drug, an alternative method of administration is daily intravenous infusion. However, this method increases the risk of infections at the site of administration and could lead to systemic toxicity, in the case of chemotherapeutic drugs for the treatment of cancer.

In order to address these problems, sustained drug delivery devices were developed, in particular, emulsions, liposomes, biodegradable microspheres and micelles. These devices have a range of shortcomings, such as poor stability of the device in the body, the use of organic solvents to incorporate drugs and low drug loading levels. These limitations have restricted the use of these devices in the delivery of drugs. In fact, these shortcomings are particularly severe for the delivery of peptides and proteins, making them unsuitable for the delivery of this class of therapeutic drugs.

Recently, there has been increased development in the synthesis and/or isolation of peptides for therapeutic uses. There are various examples, such as glucagon-like peptide-1 (GLP-1) for the control of diabetes, ghrelin for the treatment of obesity, gastrin-releasing peptide used in cancer treatments and defensin, which can be used as an antimicrobial agent. The creation of numerous peptide libraries has exponentially increased the number of therapeutic peptides discovered.
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However, the delivery of peptides to humans remains a problem owing to their short residence half-life, due to their rapid renal clearance. In order for peptide therapy to work, a sustained peptide delivery system has to be developed. Biodegradable injectable thermogelling copolymers are suitable candidates as peptide delivery agents. This is due to, and not limited to, the following reasons: i) the formulation of the peptide/polymer mixture can be done in an aqueous environment, sidestepping the use of organic solvents, which could denature the peptide; ii) the method of administration is via the injection route, which removes the need for surgical implantation of the sustained release device; iii) the system is biodegradable and is removed from the body via the natural excretion route after its intended purpose is achieved; iv) such gels are easily sterilised via syringe filtration; v) the high water content of the gel matrix means improved biocompatibility with the site of application; and vi) the rate of sustained drug release can be easily controlled by adjusting the composition of the formulations.

The objective of this review is to provide a comprehensive summary of the different types of biodegradable thermogelling copolymers in both the literature and in intellectual property. This review tries to incorporate all known examples of synthetic thermogelling copolymers, which means that natural polymers, such as gelatin and chitosan, are excluded from this review. The discussion that follows will highlight some of the applicable studies done with the thermogelling copolymers, such as drug delivery and in vivo studies of these copolymers.

2. Biodegradable thermogelling copolymers

Biodegradable thermogelling copolymers exhibit a phase change behaviour of sol-to-gel-to-sol, sol-to-gel or gel-to-sol transition upon an increase in temperature (Figure 1). The formation of the gels takes place via physical crosslinking between the copolymers. Physical crosslinks are not permanent bonds and can be formed and removed with changes in temperature. When the physical crosslinks are formed, the water is entrapped within the polymer matrix, forming a hydrogel. The phase transition can be adjusted by changing different parameters, such as composition of the copolymer and the molecular weight of the copolymer. The sol-to-gel transition is particularly attractive for applications because the drug can be mixed with the aqueous copolymer solutions at low temperatures and be injected into the body, where the higher body temperature would lead to the formation of a gel depot for the sustained release of the drug via diffusion of erosion of the copolymer gel. Thermogelling copolymers that have been surveyed in this review have different molecular architectures such as diblocks, triblocks, graft copolymers and star copolymers. These different architectures lead to the formation of different types of nanostructures in the aqueous solutions and consequently lead to different gelation mechanisms.

2.1 Modified poly(ethylene glycol)/poly(propylene glycol) copolymers

There have been many examples in literature citing the short in vivo residence time and the poor mechanical properties of Pluronics™ F127 (poly(ethylene glycol) [PEG]-poly(propylene glycol) [PPG]-PEG; BASF) gels. In order to improve the mechanical properties of these gels, the approach of increasing the molecular weight of the copolymers was utilised. A 30 wt% Poloxamer™ P85 (PEG20k- PPG40k-PEG20k; BASF) gel has an in vitro stability of 8 hrs. A chain extension of P85 was carried out by coupling with terephthaloyl chloride, resulting in a biodegradable multiblock copolymer with a molecular weight of 31,000 [1]. The 30 wt% copolymer gel showed very good stability in the erosion tests performed, persisting for several weeks. Results show that only ~ 20% of the gel eroded after 20 days. The critical gelation concentration (CGC) of the copolymers also decreased with increasing molecular weight. However, the nature of the coupling agent is a very important factor to consider when synthesising biodegradable Poloxamer blocks. Cohn et al. capped Pluronics F127 with short poly(ester) blocks by the ring opening polymerisation reaction between the end hydroxyl groups of Pluronics F127 and various lactones (lactide and ε-caprolactone) [2]. The pentablock precursors were then chain extended using hexamethylene diisocyanate. It was observed that the ability of the pentablocks to form gels was reduced when the poly(ester) end groups became larger. In particular, when the degree of polymerisation of polylactic acid (PLA) on either end was > 12, a thermogelling effect was not observed. This was attributed to a hindrance of the nanostructures in the aqueous solutions leading to a reduced tendency of the polymers to pack together to form a gel. However, the chain extension of the pentablocks restored the tendency of the copolymers to gel. Erosion studies demonstrated that the gels had enhanced stability as compared with the Pluronics F127 gels. In another related development, multiblock Pluronic F127 copolymers having either carbonate [201], urea [201] or urethane bonds [3,202] were developed. The in vitro release of RG-13577, an antirestenosis agent, shows that the gels derived from the chain extended Pluronic F127 released the drug at a rate 5 times slower than the Pluronics F127 gels [3]. Pluronics F127 were end-functionalised with acrylate groups to allow for further crosslinking upon injection [203]. Biodegradable oligoester blocks have also been introduced between the Pluronics F127 and acrylate groups, resulting in a biodegradable crosslinked hydrogel with tunable degradation rates [4-7]. High drug loading levels of the microgels were reported for haemoglobin, bovine serum albumin and insulin. In another approach to develop mechanically stronger gels, ethoxysilane-capped PEG-PPG-PEG triblocks were synthesised [8,204,205]. These gels underwent a sol-to-gel transition and then further crosslinking in situ due to the condensation reaction of the silane end groups. These gels
were tested for their drug delivery capabilities using methylthioninium chloride and metronidazole as model drugs [8]. While the Pluronics F127 gel delivered the entire drug load over slightly > 2 days, the ethoxysilane-capped PEG-PPGPEG triblock gels took 13 days to deliver the entire drug load. The metronidazole release showed a similar trend. The Pluronics F127 gel delivered metronidazole over 2 days, while the ethoxysilane-capped PEG-PPG-PEG triblock gels took 7 days. Thermogelling PEG/PPG multiblock copolymers having enhanced rheological properties when compared with Pluronics F127 were synthesised by covalently binding the two components using carbonyl chloride and diacyl chlorides as the coupling molecules [9,10,206]. The effect of alternating coupling and random coupling of PEG and PG showed that alternating PEG/PPG copolymers displayed a higher viscosity as well as a higher sol-to-gel transition temperature as compared with the randomly coupled PEG/PPG counterparts. When the molecular weight of the polymers is too low, a thermogelling effect was not clearly observed, whereas for higher molecular weight copolymers, a sharp increase in viscosity was observed at the sol-to-gel transition temperature. In order to make the copolymers biodegradable, very short oligo(ester)s were inserted into the PEG/PPG backbone. Chain extension of PEG-PPG-PEG triblock copolymers using terephthalic anhydride as the coupling agent led to the development of a high molecular weight PEG/PPG multiblock copolymer with free carboxylic acid functional groups along its backbone [11]. This copolymer forms a pH and thermo-sensitive hydrogel in aqueous solution. The resulting thermogel that is formed is stable at pH 4 – 6. Different parts of the human body have a different local pH environment in the range of pH 1 – 8. This demonstration illuminates the strategy for the development of pH-sensitive thermogelling copolymers for a specific-site application. A PEG-PPG-PEG disulfide multiblock copolymer was reported as a new reverse thermogelling polymer [12]. The higher molecular weight of the thermogelling copolymer led to a more sustained drug release of paclitaxel. A triggered release of paclitaxel was observed by exposure to a reducing environment that cleaved the disulfide bonds and degraded the copolymer gel.

2.2 Poly(D,L-lactic acid-co-glycolic acid) orpoly(L-lactic acid)/poly(ethylene glycol) copolymers

PLA is a biodegradable material that is becoming increasingly popular in the fabrication of medical devices. The basic component of PLA is lactic acid, which can be obtained from renewable resources, such as corn starch, making it an attractive candidate for incorporation in biomaterials. PEG-poly(L-lactic acid) (PLLA) diblock copolymers showed a gel-to-sol transition upon an increase in temperature [13-15,207-209]. The gel-to-sol transition temperature can be adjusted by changing the concentration of the copolymer in solution or changing the composition of the diblock copolymer. For diblock copolymers of having the same PEG segment length, longer PLLA segments led to a decrease in the CGC and a widening of the gel window. On the other hand, for diblock copolymers having the same PLA segment length, longer PEG segments led to an increase in the CGC as well as a narrowing of the gel window. Triblock PEG-PLLA-PEG copolymers exhibit a gel-to-sol transition [16,17]. The sustained release characteristics of these gels were studied and the authors predict that a month long release for protein drugs was feasible. The above examples described gels that undergo gel-to-sol transitions. This may not be suitable for the entrapment of protein drugs where the increase in temperature could cause the denaturation of the proteins. In addition, the injection of a hot formulation to the body is not a patient-friendly approach to drug delivery. PLA-PEG-PLA triblock copolymers were claimed by Macromed, Inc. [210]. These triblock copolymers were made of PEG with a molecular weight of 3400, flanked by PLA with a molecular weight of 500 – 1500 on either side. These copolymers showed a sol-to-gel transition with an increase in temperature. Recently, short alternating PEG/PLLA multiblock copolymers made up of short PEG and PLLA chain segments showed reverse thermal gelation behaviour, the gels were soluble at low temperatures and formed gels at higher temperatures [18]. When the hydrophobic segment was decreased in length, it was observed that the gel window decreased and the critical gelation concentration decreased. Increasing the molecular weight of the polymer decreased the sol-to-gel transition temperature by 2 – 4°C. This could be
due to the decreased solubility of higher molecular weight copolymers, resulting in the transition at lower temperatures. Li et al. found that when PEG/PLLA multiblocks made up of longer PEG segments were used, gel-to-sol transition behaviour was observed [19]. These multiblock copolymers had higher molecular weights than the multiblock copolymers reported by Jeong et al. These results demonstrate that the molecular weights and the segment lengths are factors that must be carefully considered when designing a degradable thermogelling polymer. A slight change in these parameters could mean the difference between a thermogelling (sol-to-gel transition) and a thermo-liquifying (gel-to-sol transition) polymer.

PLLA and poly(D,L-lactic acid) (PDLA) are hydrophobic polymers that readily crystallise, forming precipitates in solution. Thus, it is not very easy to design a water-soluble thermogelling polymer using pure PLLA or PDLA as the hydrophobic segment. In order to circumvent this problem, poly(D,L-lactic acid-co-glycolic acid) (PLGA) was synthesised by a copolymerisation process of lactide and glycolide. Poly(glycolic acid) is more hydrophilic than PLA and the copolymisation of these two components reduces the hydrophobicity of the polymer segment when compared with a pure PLLA segment. Hydrogels containing PEG and PLGA segments have been extensively investigated since 1999 and represent the most popular subject of study in the field of biodegradable thermogelling copolymers. PEG-PLGA copolymers of various molecular architectures have been synthesised and characterised. These copolymers have been studied for their micellar, gelation, drug release and biodegradation properties.

PEG-PLGA diblock copolymers were developed and shown to possess thermogelling properties in solution [211,212]. Jo et al. showed that the use of thermogelling diblock PEG-PLGA copolymers reduced the burst effect of release of FITC-dextran when compared with the triblock counterpart [211]. Chang et al. synthesised a PEG-PLGA diblock copolymer end-modified with an alkyl group. Interestingly, his group also showed that the use of diblock copolymers produced a more sustained release compared with the corresponding triblock. The end-modified copolymers achieved a release rate that was intermediate between the release rates of the diblock and triblock copolymers.

PEG-PLGA-PEG triblock copolymers were synthesised by Jeong et al. in 1999 [20]. These copolymers were water soluble at low temperatures and transformed into a semisolid gel state at elevated temperatures. In addition, the triblock copolymers showed good blood compatibility [21]. Solution properties of the copolymers were studied and the gelation mechanism was attributed to the aggregation of the micelles at elevated temperatures [22-24]. In vitro drug release studies were performed with model hydrophilic (ketoprofen) and hydrophobic (spironolactone) drugs [25]. This gel formulation possesses hydrophilic and hydrophobic domains, thus it would be suitable for the release of either type of drugs. A sustained release period of 2 weeks was observed for ketoprofen and 2 months for spironolactone. This was attributed to the different mechanisms of release for the different types of drug. In addition, these gels demonstrated excellent mechanical property and subcutaneously injected gel depots were found to last for more than a month in rats [26]. The authors report that there was a low extent of tissue irritation observed at the injection site even after 1 month. PEG-PLGA-PEG copolymers were blended with Lipiodol® (Guerbet, Aulnay-sous-Bois, France) to constitute thermogelling emulsions. Sustained release of paclitaxel was demonstrated and vascular embolisation was demonstrated in vivo [27,213]. In vivo sustained intravesical drug delivery was demonstrated using fluorescein isothiocyanate (FITC) and misoprostol. Free FITC was cleared from the animals after 8 hrs, whereas FITC delivered from the thermogelling formulation was sustained for up to 24 hrs after instillation [28]. PEG-PLGA-PEG gels were explored for potential tissue engineering applications. These gels were used to prepare biomimetic hydroxyapatite (HAp), resulting in the observation of regular plate-like shapes and nanosized hydroxyl apatite structures [29]. Wound healing is another area that bears potential application for these copolymers. In order to accelerate diabetic wound healing, TGF-β1 was entrapped in a PEG-PLGA-PEG thermogelling formulation. Application of the plasmid-loaded gel paste accelerated re-epithelialisation and increased cell proliferation in the wound bed when compared with plasmid-loaded Humatrix® [30]. PEG-PLGA-PEG hydrogels were used to deliver luciferase pDNA to skin wounds on mice [31]. In vitro studies showed zero order kinetics of supercoiled pDNA release over 12 days. After 24 h, maximal gene expression of luciferase was observed. These results suggest that thermogelling copolymers could be used as a novel non-viral gene delivery system for therapies of skin disorders and wound healing.

PEG-PLGA-PEG-PG-PEG-PLGA, carboxylic acid end-modified PLGA-PEG-PLGA triblock copolymers and the non-modified triblock copolymers were developed by Amgen, Inc. for the release of leptin for potential weight-loss treatments [214,216]. In vitro release studies showed that the integrity of the released leptin was maintained. Sustained in vivo release of leptin in mice resulted in sustained weight loss in mice over a period of 5 days. PLGA-PEG-PLGA triblock copolymers (ReGel®) were developed in 1999 by Macromed, Inc. [217-220]. These water soluble copolymers formed gels at concentrations of 5 – 30 wt% [32]. Extensive drug release studies were carried out on this promising product [33,34]. The drugs include paclitaxel, insulin, rh-GH, granulocyte colony-stimulating factor (G-CSF) and recombinant hepatitis B surface antigen (rHBsAG). The gel depots were injected in rats and found to be completely resorbed from the injection site within 4 – 6 weeks. Paclitaxel is an established chemotherapeutic for the treatment of solid tumours. In vitro paclitaxel release from the copolymer gel attained a sustained release of 50 days. The drug depot was administered in the pig pancreas using an ultrasound-guided injection technique to show the
feasibility of such a minimally invasive cancer treatment procedure [35-37]. The results show that high and localised concentrations of paclitaxel were observed at the injection site. This study proves that this formulation can be applied as a potential minimally invasive local treatment option for unresectable pancreatic tumours.

The formulation of ReGel and paclitaxel constitutes the novel intratumoural injectable, OncoGel™ (Protherics). OncoGel is designed to release paclitaxel into the tumour at a sustained rate over 4 – 6 weeks in order to achieve a greater concentration of paclitaxel in the tumour compared with that achieved when administered intravenously at the maximum tolerated dose. OncoGel is presently being explored for the treatment of oesophageal and primary brain cancers [301]. A Phase I study was conducted to characterise the toxicity, pharmacokinetics and preliminary antitumour activity associated with the direct injection of OncoGel into solid tumours [38]. Systemic side effects are minimised, as evidenced by low observed systemic levels of paclitaxel. OncoGel is in Phase Iia development for the treatment of dysphagia (difficulty in swallowing) in patients suffering from oesophageal cancer. A Phase Iib study, during which the safety and efficacy of OncoGel is to be studied, is planned to start by the first half of 2008.

OncoGel may also be used in the treatment of primary brain cancers. Treating brain cancer is extremely challenging due to the inability of most chemotherapeutic agents to cross the blood–brain barrier. The treatment protocol of the most malignant form of brain cancer, glioblastoma multiforme (GBM), has been proposed. The first approach involves the injection of OncoGel after surgical removal of the tumour into the tumour cavity to kill residual tumour cells. Another approach is targeted at patients with inoperable tumours, where the formulation will be injected directly into the tumour. Very encouraging results have been obtained from non-clinical models of brain cancer. At present, the safety and tolerability of OncoGel for the treatment of primary brain cancer is being investigated.

Besides paclitaxel, PLGA-PEG-PLGA triblock copolymer gels were also shown to deliver ganciclovir (GCV), a drug used in the treatment of human cytomegalovirus (HCMV) retinitis [39]. GCV was encapsulated in PLGA microspheres. These microspheres were then dispersed in the free flowing polymer solution. This formulation forms a gel upon heating to form a matrix structure for the sustained release of GCV over a period of 4 – 5 weeks. Kim et al. showed the controlled release of insulin from a ReGel/insulin formulation for the treatment of Type 1 diabetes mellitus, an ailment that requires daily injection of insulin [40]. Sustained release of insulin was observed over a period of 2 weeks in in vitro experiments. The sustained insulin release kept blood glucose levels in the euglycaemic range, which was observed in Zucker diabetic fatty (ZDF) rats over a 2-week period. It was concluded that this formulation was able to maintain basal insulin levels over a week upon a single injection. In another related study, the group demonstrated GLP-1 release from ReGel formulation in vitro and in vivo [41]. This peptide is used in the treatment of Type 2 diabetes mellitus. The results showed no initial burst and a constant release for 2 weeks. Animal studies conducted on diabetic rats demonstrated that the plasma insulin level was increased and the blood glucose level was controlled for 2 weeks by one injection of ReGel/ZnGLP-1 formulation. The authors concluded that one injection of zinc-complexed GLP-1 loaded ReGel can be used for delivery of bioactive GLP-1 over a 2-week period. This new biocompatible delivery system was proposed as a highly attractive alternative for the delivery of GLP-1 as it can improve patient compliance and result in cost-effectiveness in the treatment of diabetes. PLGA-PEG-PLGA triblock copolymer gels can be used to deliver a variety of bioactive agents, such as levonorgestrel, cefazidime, testosterone, bee venom peptide, 5-fluororacil, IL-2, indometacin and lysozyme [42-47]. The use of these copolymers as a non-viral vector for targeted gene delivery has also been studied [48-50]. Kim et al. also fabricated biodegradable microspheres using these triblock copolymers [51]. In one set of experiments, insulin was entrapped within the PLGA-PEG-PLGA microspheres without the use of organic solvents (Msp A). In another set of experiments, the microspheres were made using the conventional method using organic solvents (Msp B). The sustained release of insulin from Msp B showed an initial burst and incomplete release, whereas Msp A showed a relatively sustained release of 10 days.

An often cited weakness in the PLGA-PEG-PLGA and PEG-PLGA-PEG triblock systems is the high concentrations of polymer required in the aqueous solution for the thermogelling effect to be observed. In order to enhance the gelation behaviour and lower the thermogelling concentrations, an end-capping approach was adopted to tune the hydrophobic/hydrophilic balance of PLGA-PEG-PLGA copolymers [52,53]. By incorporating diacetate or dipropionate end groups into the triblocks, it was observed that the critical gelation concentration was significantly decreased. The addition of the end groups in the triblocks enhanced the hydrophobic interactions between the micelles such that there is sufficient driving force to induce the large-scale self assembly of micelles.

PLGA-g-PEG and PEG-g-PLGA were developed in an attempt to overcome the molecular weight constraint of the triblock copolymers [54-56,221,222]. Interestingly, injected gel deposits of PLGA-g-PEG lasted for a period of 3 months, whereas PEG-g-PLGA lasted for < 1 week. By mixing different compositions of these copolymers, the duration that the gel is able to last in the body can be varied for implantation applications from between 1 week and 3 months [57]. These polymers were studied for potential protein and cell-based therapy through in vivo biomedical applications using animal models, particularly for diabetic control by sustained insulin delivery and cartilage repair by chondrocyte cell delivery [58].
Upon standing at 20 °C overnight, a previously free-flowing copolymer in aqueous solution exhibits a unique behaviour with an increase in temperature.

PEG-PCL-PEG and PCL-PEG-PCL triblock copolymers were synthesised and found to undergo a sol-to-gel transition upon a temperature increase [68,69]. The advantage provided by this system as compared with the PLGA-based thermogelling copolymers is that the incorporation of PCL leads to the formation of polymers in powdery form, instead of a sticky paste. This leads to easy handling of the drug/polymer formulation for practical applications. PCL-PEG-PCL triblock copolymers exhibit a unique behaviour in aqueous solution. Upon standing at 20 °C overnight, a previously free-flowing solution forms a gel. This was attributed to the crystallisation of the PCL blocks in the polymer formulation. Multiblock PEG-PCL copolymers were previously synthesised and found to undergo a gel-to-sol transition upon temperature increase [70]. The molecular weights of these polymers were in the range of 16,000 – 70,000. Shorter PEG-PCL multiblock copolymers were found to undergo a sol-to-gel transition as the temperature increased [71]. Additionally, these multiblock copolymer solutions were stable as a transparent solution at room temperature, providing practical convenience during the drug formulation.

Caprolactone has been copolymerised with lactide and glycolide to form PCLA and PCGA, respectively, and incorporated into PCLA-PEG-PCLA and PCGA-PEG-PCGA triblock copolymers [72,74]. These triblocks were then further end capped with the sulfamethazine oligomers to yield pH- and temperature-sensitive hydrogels. The degradation behaviours of the gels were studied and OSM-PCLA-PEG-PCGA-OSM copolymer gels were found to degrade at a much faster rate than OSM-PCLA-PEG-PCLA-OSM copolymer gels [75]. In vivo biocompatibility studies of OSM-PCLA-PEG-PCLA-OSM copolymer gels were performed by subcutaneously injecting the gel into Sprague–Dawley rats. Apart from a mild hyperemic erythema detected at the injection site, other adverse reactions, such as oedema formation, haemorrhaging and discolouration, were not observed during the first 6 weeks. After 6 weeks, the hydrogel had completely degraded in vivo and the affected region was fully covered with connective tissue. These results indicate that OSM-PCLA-PEG-PCLA-OSM copolymer gels are fully biocompatible in vivo and, thus, suitable for use in drug delivery systems. In vivo release of paclitaxel was performed with OSM-PCLA-PEG-PCLA-OSM copolymer gels and showed good antitumour effect for 2 weeks and induced strong apoptosis in tumour tissue [76]. In a separate development, PCLA-PCL copolymers, which were water-soluble but did not possess the thermogelling property, could be aliphatically modified with a hexanoyl or lauroyl group to form a thermogelling copolymer [77].

2.3 Poly(ε-caprolactone)/poly(ethylene glycol) copolymers

Poly(ε-caprolactone) (PCL) is a hydrophobic and crystalline polymer that shows good biocompatibility and has been used in the fabrication of many biomedical related materials. Diblock PEG-PCL copolymers with PEG segment lengths of 750 and PCL segment blocks of 1400 – 3000 were soluble in water and underwent a sol-to-gel transition at room temperature increase [61-62]. PEG-PCL diblocks having PEG segment blocks of 2000 and PCL segment length of between 950 – 1500 underwent a gel-to-sol phase transition as the temperature was varied [63-65]. The in vivo differentiation of osteogenic differentiation of rat bone marrow stromal cells (BMSCs) was investigated. These in situ forming gel scaffolds containing dexamethasone enabled rBMSCs were found to generate viable bone when injected into rats [66,67]. These PEG-PCL-PEG and PCL-PEG-PCL triblock copolymers were synthesised and found to undergo a sol-to-gel transition with an increase in temperature [68,69,210]. The advantage provided by this system as compared with the PLGA-based thermogelling copolymers is that the CGC of the copolymer could be reduced by increasing the lactide content relative to the glycolide content. This finding appears to imply that a higher hydrophobic content leads to a lower CGC in such star-shaped copolymers.

Recently, the synthesis and thermogelling behaviour of biodegradable poly(D,L-3-methyl glycolide)-block-poly(ethylene glycol)-block-poly(D,L-3-methyl glycolide) triblock copolymers were reported [60]. The synthesis of this copolymer was done with a biocompatible Ca-based catalyst system. In addition, the copolymers formed have a uniform molecular structure of alternating lactyl and glycolyl units instead of a blocky microstructure with a gradient distribution of lactyl and glycolyl units throughout the chain.
very low copolymer concentrations (2 – 5 wt%). As a result of its multiblock architecture, a novel associated micelle packing model can be proposed for the sol-to-gel transition for the copolymer gels of this system. A thorough protein drug release and degradation study of the copolymers was carried out [79]. The gels showed a sustained protein release of > 70 days. The copolymer gels degraded slowly over a period of 6 months, making it stable for long-term applications. This new material is thought to be a promising candidate as an injectable drug system that can be formulated at low temperatures and forms a gel depot in situ upon subcutaneous injection.

2.5 Poly(organophosphazenes)

Biodegradable thermosensitive poly(organophosphazenes) bearing a short hydrophilic PEG segment, hydrophobic amino acid esters (such as D,L-leucine [LeuOEt] ethyl ester, L-isoleucine ethyl ester [IleOEt] and L-valine ethyl ester [ValOEt]) and a depsipeptide ethyl ester (ethyl-2-[O-glycyl]glycolate) as a hydrolysis-sensitive moiety were synthesised by Song et al. [80,81,223]. Seong et al. reported the synthesis of similar poly(organophosphazenes), except that the hydrophobic amino acid segment was made up of tripeptides or tetrapeptides (such as GlyPheLeuEt, GlyPheIleEt, GlyLeuPheEt and GlyPheLeuGlyEt) [82]. The poly(organophosphazenes) showed sol-to-gel transition properties in an aqueous solution at concentrations of 10 wt%. The main driving force for the thermosensitive gelation of poly(organophosphazenes) is the temperature-triggered intermolecular association between hydrophobic amino acid ester side groups. When the amino acid side groups were the ethyl esters of glycine, β-alanine, alanine, L-aspartic acid and L-glutamic acid, the copolymers were not reported to form thermoreversible hydrogels [83]. Instead, aqueous solutions of the copolymers showed sol-precipitation behaviour upon exposure to a monotonic increase in temperature. The gelation properties of these gels could be easily tuned by adjusting the composition of substituents, the chain length of the PEG segment attached and the type of amino acid ester segment attached to the poly(organophosphazene) backbone [84]. In degradation studies, the molecular weight of the polymer was found to decrease by 60% at 37°C over 2 months [81]. The biodegradability of the polymer can be controlled by varying the amount of depsipeptide incorporated. The degradation of the depsipeptide generates free carboxylic acid groups that, in turn, catalyse the degradation of the amino acid side groups. Inorganic and organic salts were found to influence the thermogelling behaviour of the poly(organophosphazenes) [85]. For example, the addition of sodium bromide to a gel formulation led to a six times increase in the maximum attained viscosity of the gel. The hydrogels were tested for drug release properties and release of human serum albumin and FITC-dextran were demonstrated [86]. For chemotherapeutic treatments, the poly(organophosphazene) hydrogels were found to be excellent solubilisers of the hydrophobic anticancer drug, doxorubicin [87]. The hydrogel system maintained a sustained release of doxorubicin over 20 days. Anticancer efficacy of doxorubicin released from the hydrogel was evaluated using the P388D1 mouse lymphoblast cell line. It was concluded that doxorubicin released from the polymer hydrogels retained antitumour activity for > 30 days. Poly(organophosphazene)s bearing ω-amino-ω-methyl-poly(ethylene glycol) (AMPEG) and hydrophobic L-isoleucine ethyl ester (IleOEt) side groups were dissolved in a buffered solution at 10 wt%. This thermogelling formulation was used to entrap islets of Langerhans in an artificial pancreas [88]. Rat islets entrapped in the gel showed prolonged insulin secretion in response to basal glucose concentration compared with free rat islets and islets entrapped in other types of polymer gels. Over a 28-day culture period, the rat islets in the poly(organophosphazene) hydrogel maintained higher cell viability and insulin production when compared with rat islets in different hydrogels and free islets. From this demonstration, the authors proposed that the thermogelling poly(organophosphazene) can be used as an injectable and biodegradable matrix. In another study, the morphology of primary rat hepatocytes cultivated as spheroids and entrapped in a poly(organophosphazene) hydrogel matrix was examined for differentiation morphology and enhanced liver-specific functions [89]. In the 28-day culture period, the spheroidal hepatocytes in the gel matrix maintained higher viability and albumin and urea production was found to be at constant rates. This demonstration shows the potential of using the poly(organophosphazene) hydrogel system as a three-dimensional cell system for application in bioartificial liver devices and bioreactors.

2.6 Poly(propylene fumarate)/poly(ethylene glycol) copolymers

Triblock copolymers with ABA-type block structure were synthesised from poly(propylene fumarate) (PPF) and mPEG utilising a simple transesterification procedure [224]. ABA copolymers synthesised with mPEG of average molecular weight 570 and 800 showed thermoreversible properties that were dependent on the mPEG molecular weight and salt concentration [90]. In vitro release of TGF-β1 from gelatin microparticles entrapped in these hydrogels were reported [91]. PPF is a biodegradable hydrophobic polyester which has been thoroughly characterised and investigated for potential orthopedic applications. PPF consists of propylene fumarate units and can be crosslinked in situ via its fumarate double bonds. The copolymerisation of PPF with PEG resulted in a hydrophilic, biodegradable, biocompatible and in situ crosslinkable copolymer. This class of polymers could find future applications for the delivery of a pharmaceutically active agent to the eye [225,226]. Specifically, the sustained release of an anti-inflammatory drug, flucinolone acetonide, was demonstrated. However, unlike the other thermogelling systems, these copolymers are often further
crosslinked after application to the biological site in the demonstrations.

### 2.7 Poly(peptides)

Polypeptides and poly(amino acids) are important biomedical materials due to their favourable biocompatibility and biodegradability. The synthesis of high molecular weight polypeptides through synthetic chemistry methodologies is expensive. However, scientists can take advantage of the genetic engineering of cells to produce a polypeptide of a defined sequence. Polypeptides can be tailored to fold in various conformations and the building blocks of amino acids have different characteristics (hydrophobic, hydrophilic, cationic, anionic). These favourable factors open an enormous door of opportunities for material scientists to exploit. An artificial trilobed protein consisting of leucine zipper end blocks flanking a water-soluble polypeptide domain were reported to undergo a thermosensitive gel-to-sol transition when the temperature was raised [92]. Gelation of the protein is driven by the formation of coiled-coil aggregates of the terminal domains. The middle polypeptide segment acts to retain the solvent in the network structure and prevent the formation of precipitates. Thermosensitive hybrid polymers made of synthetic water soluble polymers and protein blocks were reported [93]. The protein blocks act as crosslinks that undergo folding transitions with changes in temperature. Macroscopic volume transitions were observed when the hydrogel was heated. Thermally reversible physically crosslinked poly(amino acid) hydrogels can be formed by the sol-to-gel transition of amphiphilic poly(N-substituted \( \alpha/\beta \)-asparagine)s in an aqueous solution [94]. These poly(amino acid)s showed a sol-to-gel transition even at a 3% concentration. Another thermosensitive gel derived from polypeptides containing a collagen-derived Pro-Hyp-Gly sequence and an elastin-derived Val-Pro-Gly-Val-Gly was reported [95]. The collagen model peptide acts as a hydrated unit and the elastin-derived pentapeptide acts as a thermosensitive crosslinking point. These gels also require very low concentrations for physical gel formation to occur. The authors propose a potential application of incorporating functional peptides to enhance the cell adhesion properties of these gels. Jeong et al. have reported pH- and temperature-responsive poly(aspartic acid)-g-poly(propylene glycol) [96].

### 2.8 Others

Many other types of systems have been reported to form thermogelling copolymers. Kim et al. reported the synthesis of thermogelling diblock copolymers consisting of PEG and hydrophobic segments made up of caprolactone and either trimethylene carbonate (TMC) or 1,4-dioxan-2-one (DO). This method uses HCl as a monomer activator, which is a safer alternative to the known cytotoxic stannous-based catalysts [97,227]. Jeong et al. reported that multiblock PEG/sebacic acid copolymers were synthesised and formed thermogelling formulations [98]. Rapid formation of the gel was observed upon exposure to higher temperatures. The sol-to-gel transition of these copolymers takes place via a packing of the polymer chains instead of an aggregation of micelles, as reported for other classes of copolymers. The thermoreversible gelation of triblock copolymers of PEG and aliphatic polyesters (poly(hexamethylene adipate) [PHA], poly(ethylene adipate) [PEA] and poly(ethylene succinate) [PESc]) were reported [99]. These polymers underwent a gel-to-sol transition with increasing temperature. PEG-PHA-PEG and PEG-PEA-PEG triblock copolymers needed high polymer concentrations to form gels, whereas PEG-PESc-PEG copolymers showed gelation at a low polymer concentration. This was attributed to the difference in hydrophobicity of the polyester blocks. A more hydrophobic core resulted in the formation of a more compact (and smaller) micelle core, thus a higher critical micelle volume (higher critical gelation concentration) was required to induce gelation.

A stimuli responsive hydrogel based on poly(propylene phosphate) was synthesised [100]. This hydrogel shows temperature induced sol-to-gel transition in the presence of calcium ions. In vitro release of plasmid DNA and lysozyme was demonstrated. This report states that this system can be applied as an injectable material for controlled drug and gene delivery. PEG-polyacetal-PEG triblock copolymers and PEG-poly(ortho ester) graft copolymers were reported to form thermogelling copolymers [101,228,229]. These PEG-polyacetal-PEG triblock copolymer gels were shown to provide sustained release of ondansetron (7 days) and paclitaxel (50 days).

### 3. Conclusions

The past decade has seen the development of many novel thermogelling copolymers having potential applications in the areas of sustained drug delivery, gene delivery and tissue engineering. Most of these thermogelling copolymers are still at developmental research stage. So far, there has only been one example of a commercially viable thermogelling copolymer/drug formulation (OncoGel). This formulation is in the advanced stages of clinical trials and its launch can be expected in the very near future. This product can be used for a minimally invasive procedure for the cancer treatment.

### 4. Expert opinion

At present, the area of biodegradable thermogelling copolymers is at a developmental stage and one would expect that many researchers would be laying claims to their inventions. Before a decision can be made on the use of the copolymers for biomedical applications, several questions must be addressed: what is the duration of use? What happens to the polymer after use? Are these copolymers and their resulting degradation products safe for use in the body? From these considerations, research in biodegradable thermogelling copolymers can be envisaged to grow in the following areas: i) development of novel thermogelling copolymers;
ii) fundamental structure–property relationship studies; iii) lifetime or degradation studies; and iv) \textit{in vivo} applicative studies, such as drug and gene delivery.

The research area of biodegradable thermogelling copolymers for biomedical applications is a very wide field of study. It is unlikely that any one synthetic copolymer will fulfil the requirements of all the different applications. For this reason, new materials have to be developed for specific applications. The biological interaction between the material and the body is an important factor to consider when using these gels in the body. For example, functionalisation of the copolymer with cell-adhering components could improve the attachment of the gel to the injection site.

In the area of sustained release, the period of release can be extended for long-term applications. Parameters, such as molecular weight and hydrophobic/hydrophilic balance, have been used to fine-tune the gelation properties of the copolymers. Copolymers of different molecular architecture could be developed to make different types of gels, \textit{viz.} soft gels for short-term applications and robust, hard gels for long-term applications.

The degradability of the polymer should be assessed in reasonable depth. This will allow researchers in this field to have a better understanding of the lifetime of the product, the various degradation mechanisms and the nature of the degradation products. To date, there have not been many studies carried out to determine the degradation products and the degradation mechanism. Existing thermogelling copolymers incorporate polyesters as the hydrophobic component in the copolymers. Polymers having PLGA and PLA components are suitable for short-term applications. For long-term applications, copolymers having hydrolytically less liable bonds would be preferred. For this reason, copolymers can be incorporated with materials that are known to degrade slowly \textit{in vivo}, such as PCL, PHB, poly(peptide)s, poly(urea)s and poly(urethane)s. The toxicity of the degraded fragments remains a major concern. The degraded products of polyesters are acids, which could lower the pH of the surrounding environment, leading to potential inflammation of the injection site. Therefore, instead of using polyesters, other alternatives could be explored for use as the hydrophobic component in the copolymers.

\textit{In vivo} experiments have already been conducted for many of these thermogelling copolymers. Future studies could focus on the circulation and ultimate fate of the degradation products in the body as well as the efficacy of the drug after a long period of sustained release. These polymers are particularly attractive when used as peptide drug delivery carriers due to its mild drug encapsulation conditions.

This field bears enormous applicative potential, particularly for cancer treatments. In the coming years, it is expected that copolymers would be further developed for use in different types of applications, such as tissue engineering, ophthalmic drug release and gene delivery, and wound healing would be developed. Injection is the major route of administration of conventional thermogelling copolymers at present. Other routes of administration, such as transdermal, inhalation or oral, could be explored. This would bring us closer to the day when humans will benefit from the applications of copolymer gels used in medical treatment and patient care.

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Biodegradable thermosensitive copolymer hydrogels for drug delivery

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**Thermosensitive hydrogel proposed for gene delivery.**


**This is the first report of polycatel and poly(ortho ester) based thermogels. Initial reports show that it could be advantageous for sustained release of drugs.**

### Patents


### Website


Protherics: OncoGel™ (2007).

### Affiliation

Xian Jun Loh1 & Jun Li1,2

1Author for correspondence

2National University of Singapore, Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602, Singapore

Division of Bioengineering, Faculty of Engineering, 7 Engineering Drive 1, Singapore 117574, Singapore

Tel: +65 6516 7273 or 6874 8376; Fax: +65 6872 3060; E-mail: bielj@nus.edu.sg