Pre-clinical and clinical applications of thermoreversible hydrogels in biomedical engineering: a review

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INTRODUCTION

Thermoreversible polymer hydrogels (TRGs) are 3D physical polymeric networks in aqueous media. These hydrogels are formed as a result of temperature variation, via physical interactions between the different polymer chains, with the physical bonds relying on hydrogen bonding, hydrophobic junctions etc. As the nature of these bonds is physical, the gelation is reversible, with the gel returning to the solution state as the stimulus, i.e. temperature, is removed, when sufficient time is allowed. However, it should be noted that irreversibility can be caused when additional interactions are introduced,1,2 such as stereocomplex formation within the gel structure3 or due to other additives interfering with the physical network.1 Depending on whether the sol–gel transition occurs as the temperature is increased or decreased, these systems are characterised by lower and upper critical solution temperature (LCST and UCST), respectively. LCST-type TRGs have received much attention due to their potential application in the tissue engineering (TE) and drug/gene delivery fields.3–5 For these applications, an aqueous solution of polymer is mixed with cells (for TE) or drugs-genomes (for delivery) at room temperature (r.t.); thus their structural integrity is preserved, and the mixture is easily loaded into a syringe. Upon injection into the body, a stable gel is formed, because of the increase in temperature to body temperature (b.t.), which traps the compounds of interest, i.e. cells and/or drugs/genes. In the case of TE, the cells proliferate and thus tissue formation occurs while the gel disappears, either via dissolution or degradation. In the drug/gene delivery field, controlled and local release takes place, thus increasing the bioavailability of the compound. The local release is beneficial especially in the case of drug delivery, as it minimises the side effects associated with the systemic release. This is shown schematically in Fig. 1. In drug delivery, epicutaneous application might take place for the treatment of skin disorders, such as acne6 and psoriasis.7 In this case, the sample is applied as a gel, while the solution phase at low temperatures facilitates the homogeneous loading of the drug.

TRGs have been widely studied during the last 50 years, with the first systematic study on TRGs with LCST behaviour dating back to 1984.8 The progress in polymer chemistry facilitated the synthesis of thermoresponsive polymers and thus a great deal of relevant research has been published.9–24 These studies aim to produce thermoresponsive polymers and investigate their LCST transition and thermogelation, with the main goal to design systems that show clear sol–gel transition between r.t. and

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Natural polymers possessing thermoresponsive properties have also been investigated as TRGs. Synthetic polymers have received much attention due to their numerous advantages, with possibly the most important one being their tailor-ability, i.e. their properties can be tuned to match the requirements of the desired application. Also, the synthesis is reproducible; thus they are characterised by 'batch-to-batch uniformity'. In addition, depending on the polymerisation method, they can be produced in a large scale at low cost, and they have long shelf-time.

Prior to in vivo application, gelation at b.t. and in vitro biocompatibility should be confirmed. In the case of degradable systems, in vitro degradability tests should also be carried out. When the TRGs are used as drug delivery matrices, the effect of the drug on the gelation properties should also studied, since it has been shown previously that the incorporation of drugs affects the gelation. As an example, when sodium chloride is added to poloxamer solutions, the gelation point decreases as the ionic strength increases, as sodium chloride is well known to cause the ‘salting-out effect’, i.e. reduction of the solubility with increased ionic strength. On the other hand, when sodium thiocyanate was used, a ‘salting-in effect’ was observed instead, with the gelation region becoming narrower and moving to higher temperatures. Therefore, the gelation in the specific system of polymer–solvent of interest should be studied prior to the application.

Previously published reviews have summarised and discussed the thermodynamics of the ‘hydrophobic effect’, the effect of the structure on the gelation, and the studies are shown in order of increased complexity of architecture (within the same category of TRG). Additional groups that are chemically bonded on the main thermoresponsive species are also presented. Any polymeric additives that are physically mixed in the gel precursor are listed, as they affect the gelation temperature and concentration.

The thermoresponsive systems, i.e. polymer structure, solvent and concentration, the model compounds used (if any) and the main in vivo results are summarised in Table 1. The thermoresponsive polymers in cell sheet engineering, and drug delivery through micellisation. The current review focuses on discussing the studies in which synthetic TRGs with LCST behaviour have been applied in vivo. These studies are categorised depending on the general structure of the polymer and/or repeated unit which provides thermoresponsive behaviour: (i) poloxamers (also known as Pluronics®), (ii) other degradable ethylene glycol (EG) based polymers besides poloxamers, (iii) poly(N-isopropylacrylamide) (PNIPAAm), (iv) poly(N-isopropylacrylamide) (PNIPAAm), (v) poly(organophosphazene)s and (v) poly(2-ethyl-2-oxazoline) (PEtOx). These units are often combined with other species in order (i) to achieve gelation at the desired temperature, (ii) to improve the mechanical properties of the gel and/or (iii) to introduce degradability into the structure. The chemical structures of these units are shown in Fig. 2. Most of the in vivo tested injectable gels show sol–gel transitions between r.t. and b.t.; thus the sol phase is loaded into a syringe and, upon injection into a model animal, a gel is formed. Nevertheless, injection of the TRGs in the gel phase was also tested, which is feasible due to the shear-thinning properties of TRGs.

Figure 1. Application of thermoreversible hydrogels (TRGs) as injectable gels in (a) tissue engineering (TE) and (b) drug/gene delivery. In both applications, the solution phase at room temperature (T_r.t. < T_gel) facilitates (i) easy mixing of the solution with cells and/or drugs and (ii) the easy loading of the mixture into a syringe. Upon injection, gelation occurs, because of the temperature increase to body temperature (T_b.t. > T_gel). In the TE application, new tissue formation takes place at the defect side, while the polymer leaves the injection side. In the drug/gene delivery concept, topical and sustained release of the molecule of interest takes place, thus increasing the bioavailability in the area of interest and minimising side effects.
Commercially available thermoresponsive polymers

- Pluronic®
- Lutrol®
- Antarox®

Other thermoresponsive components applied in vivo

- Poly(N-isopropylacrylamide) (PNIPAAm)
- Poly(organophosphazene)
- Poly(2-oxazolino)

Common monomers to introduce degradability

- L-Caprolactone (CL)
- Glycidol (GL)
- Lactic acid (LA)
- Amino acid

Figure 2. Names, chemical structures and abbreviations of commercially available thermoresponsive polymers (top), other common thermoresponsive components (middle) and monomers typically incorporated into the structure to introduce degradability (bottom).

PRE-CLINICAL STUDIES

Poloxamer based

One of the most extensively studied families of thermoresponsive polymers is poloxamers, which are ABA triblock copolymers with A and B blocks being based on EG and propylene glycol (PG), respectively. While EG is highly hydrophilic and shows a thermoresponsive phase at high temperatures, PG is thermoresponsive below \( r_g \approx 11.6 \text{kg mol}^{-1} \) thus poloxamers show temperature-driven micellization, as PG changes from hydrophilic to hydrophobic, and temperature-driven gelation in highly concentrated solutions (Fig. 3). The gelation mechanism of poloxamers has been extensively studied by small-angle X-ray and neutron scattering techniques, and the gelation is attributed to the packing of the micelles in a well-ordered structure. These polymers are commercially available, and the following registered tradenames have been identified: Pluronic®, Lutrol® and Antarox®, available from BASF; and Syneron® and Antarox®, available from Croda and Rhodia, respectively. Several poloxamers are commercially available, differing in composition and molar mass (MM), thus poloxamer gels at lower temperatures and concentrations compared to the other members of the family. The most commonly used poloxamer in TRG-related studies is poloxamer 407 (Pluronic® F127, MM \( \approx 12.6 \text{ kg mol}^{-1} \)) and EG 70 wt%\(^{5,43-56,58-105,195,196} \) as polymer additive/viscosity modifier. Nevertheless, studies on mixtures of P407 with P124, P123 and P338 have also been reported. These mixtures, loaded with drugs, were tested as drug delivery systems. Formulations based on mixtures of poloxamers have also been investigated as in vivo injectable systems. This strategy has been applied in an attempt to control the thermogelling properties of the final system as desired. In all cases, the main thermorespective component was P407, while most of the studies used P188 for drug delivery concept, solutions of P407 were investigated in vivo as carriers for HIV-1 lentiviral vector, guetiapine fumarate, latanoprost, paclitaxel (PTX), laptatinib, vancomycin, brimodine tartrate, rapamycin, halobetasol propionate, polyphenol tannic acid, dapsone, isoflavone and simvastatin. This indicates the wide variety of diseases, ranging from cancer to chronic otitis, ocular-related diseases, \(^{51,52,61} \) and wound healing, for which TRGs have been in vivo tested as drug delivery carriers. It also highlights the great potential of these gel matrices in biomedical engineering, as it has been observed that incorporation of the drugs in the gel depot provides local release and controls the drug release rate. An approach which has been proven to be effective in improving the drug bioavailability is to incorporate the drug molecules in particles, such as liposomes and niosomes, prior to their incorporation in the gel matrix.

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| Category | Architecture | Additional covalently linked species | Polymer additives | Solvent | Conc. (w/w% or w/v) | Application | Active compound | Main in vivo result | Reference |
|----------|--------------|--------------------------------------|-------------------|---------|---------------------|-------------|----------------|-------------------|-----------|
| Poloxamer-based (P407) | Triblock | — | Poloxamer | PBS, DMEM | 30 | TE | Gene, QF, PTX, lapatinib, Van, rapamycin, bromidine tartrate, polyphenol tannic acid, halobetasol propionate, dapsone, soy isoflavone, RNA polymerase inhibitor, simvastatin | Gel stability, chondrogenesis | 43, 44 |
| Poloxamer besides P407: P188, P124, P123 and P338 | H2O, PBS, NMP, BBS, STF | 19–32 | Estradiol valerate, piroxicam, DOX, PTX, astragalus, octreotide, disulfiram, Met, ketamine, duloxetine, bedaquiline fumarate, alendronate, timolol maleate, levosulpiride, SF, monosialoganglioside | Biocompatibility, ↑ bioavailability, burst or controlled release (system dependent), control of tumour | 57–73 |
| Other: PVA, MC-based, SA, Carbopol®, chitosan, sodium poly(acrylate), glycerin, polycarbophil, MPEG-PDLA, glycosaminoglycans – P188 (in some cases) | H2O, PBS | 15–40 | Octreotide, PTX, TMZ, insulin, safrarine, lacosamide, rivastigmine tartarate, S-Fu, dipivefrin, DTX, DOX, norcantharidin, TMZ, budenoside, finasteride, ciprofloxacin, carbamazepine, cinnarizine, viral vectors, lacosamide, capsazepine, betaxolol, voriconazole, atorvastatin calcium, tin protoporphyrin | Biocompatibility, ↑ bioavailability, controlled release, control of tumour | 74–101 |
| Glycosaminoglycans | H2O | 25 | TE | BMP | Injured cartilage recovery | 102 |
| Tetraaniline Graft | Chitosan | — | PBS | 18 | — | — | Gel formation | 103 |
| Pentablock tribMeCa Poloxamer-based (P105) | LA | — | H2O, NS | 35, 40 | Delivery | Antimicrobial peptides, DOX, oxaliplatin | Wound healing, control of tumour | 106, 107 |
| Other degradable EG-based | LA, CPH | — | PBS | 15–25 | — | — | Biocompatibility | 108 |
| Triblock | CL | — | H2O | 20, 30 | — | — | Controlled release | 109, 110 |

Reference: Constantinou, AP, Georgiou, TK. (2021). Polymer International. Wiley Online Library.
| besides poloxamer | H₂O, NS | 20, 25 | Delivery | Lidocaine, bFGF, CPT | Controlled release, control of tumour | 113–116 |
|------------------|---------|--------|----------|---------------------|-------------------------------------|--------|
| PLGA             | H₂O     | 20–40  | —        | —                   | Gelation, biocompatibility, degradation | 30, 117–119 |
|                  | H₂O, NS, PBS | 20–25 | Delivery | Interleukin-2, insulin, glucose-like peptides, 5-Fu, A-phospholipid, PTX (OncoGel®), irinotecan, DXA | Biodegradation, control of tumour, prevention of adhesion | 31, 40, 41, 120–127 |
| PCLA             | PBS     | 25     | —        | —                   | Degradation | 42 |
| CL, GA           | PBS     | 25     | Delivery  | COX | Controlled release | 128, 129 |
| CL, TSUN HA  | NS      | 25     | —        | —                   | — | 130 |
| ESHU             | H₂O     | 60     | —        | —                   | — | 131 |
| Peptide          | NS      | 15     | Delivery  | Bevacizumab | Controlled release | 132 |
|                  | PBS     | 8      | —        | —                   | Degradation | 133 |
| Pentablock PCLA, PLA | PBS | 25  | Delivery  | CA4, DTX | Tissue formation | 134 |
| Multiblock PG, PTHFC | H₂O      | 25 | —        | —                   | — | 135 |
| Star PCL, GLA, (M)AA, NHS, NEGMA, BuLaA, ADMBuLA, tetraamline, BuA | PBS | 25 | — Delivery | Ind | Control of tumour | 136 |
| PNIPAAm-based Random HAMA, MAA, HEMA-g-PTMeCar-Ind | PBS | 5 | TE | ADSC | Tissue formation | 137 |
| PNIPAAm ABA BuA, EG | Dextran-CL-HEMA | PBS | 8 | Delivery | BSA | Controlled release | 142 |
| Triblock ABC DMAAm, PS, CL, PLGA | H₂O, PBS | 5 | — Delivery | ADMSC, strontium, RGD peptide, hMSC, MSC, ADMSC, BMP | Osteogenic differentiation | 143 |
| Pentablock Branch QL, EG PEGC | H₂O | 20 | — Delivery | Chemokine | Tissue formation | 144 |
| Graft HA, gelatin, chitosan, MAP | PBS 5 | 10 | Delivery | Chemokine | Tissue formation | 145 |
|                | PBS     | 5–11, 55 | TE | — | Gelation | 153 |
|                | PBS     | 5 | — | — | Gelation | 154 |
|                | PBS     | 10 | Delivery | Chemokine | Tissue formation | 155–160 |
Table 1. Continued

| Conjugate          | Polyelectrolyte-based | Poly(organophosphazene)-based | Poly(ε-caprolactone)-based | PLA, poly(lactic acid-co-glycolic acid) | PEG, poly(ethylene glycol) citrate | PLGA, poly(ε-caprolactone-co-lactide) | NS1 | 10, 13 Delivery | BSA, gentamicin, pilocarpin | Vascularisation, adipose, chondro-, osteogenesis | Controlled release, antibacterial, antiallergic | TE VEGF | Vascularisation | 163, 164 |
|--------------------|-----------------------|-------------------------------|---------------------------|----------------------------------------|------------------------------------|-------------------------------------|-----|----------------|-----------------------------|-----------------------------------------------|-----------------------------------------------|---------|----------------|----------|
| H2O, PBS 10, 13    |                       |                               |                           |                                        |                                    |                                     |     |                  |                              |                                               |                                               |         |                |          |
| DOX, CPT, PTX, gene, HGH, Fe3O4 |                       |                               |                           |                                        |                                    |                                     |     |                  |                              |                                               |                                               |         |                |          |
| PVA, poly(vinyl alcohol) |                       |                               |                           |                                        |                                    |                                     |     |                  |                              |                                               |                                               |         |                |          |
| QF, quetiapine fumarate |                       |                               |                           |                                        |                                    |                                     |     |                  |                              |                                               |                                               |         |                |          |
| Dox, DOX, CPT, PTX, DTX, Fe3O4 |                       |                               |                           |                                        |                                    |                                     |     |                  |                              |                                               |                                               |         |                |          |
| PEG, poly(ethylene glycol) citrate |                       |                               |                           |                                        |                                    |                                     |     |                  |                              |                                               |                                               |         |                |          |
| DOX, CPT, PTX, DTX, Fe3O4 |                       |                               |                           |                                        |                                    |                                     |     |                  |                              |                                               |                                               |         |                |          |

Note: The studies are summarised in terms of the main thermoresponsive components and any polymeric additives (physically mixed or covalently linked). The solvent and concentration used, the targeted application, active compound (cells, drug, gene) and main in vivo results are listed.

Abbreviations: AA, acrylic acid; ADMBuLA, acryloyloxy dimethyl-γ-butyrolactone; ADMSC, adipose derived mesenchymal stem cells; ADSC, adipose-derived stem cells; Ag NPs, silver nanoparticles; BBS, borate buffer solution; tFGF, basic fibroblast growth factor; BMSC, bone marrow mesenchymal stem cells; BMP, bone morphogenetic protein; BMMSC, bone marrow mesenchymal stem cells; BSA, bovine serum albumin; BSS, balanced salt solution; BuA, n-butyl acrylate; BuLaA, γ-butyrolactone acrylate; CA4, combrestatin; CCM, cell culture medium; CL, ε-caprolactone; COX, celecoxib; CPH, 1,6-bis(p-carboxyphenoxy)hexane; CPT, camptothecin; DMAAm, N,N-dimethylacrylamide; DMEM, Dulbecco's modified Eagle's medium; DO, p-dioxanone; DOX, doxorubicin; DTX, docetaxel; DXA, dexamethasone acetate; EG, ethylene glycol; ESHU, serinol hexamethylene urethane; F-U, 5-fluorouracil; GA, glycolic acid; HA, hyaluronic acid; HAMA, hyaluronan methacrylate; HEMA, 2-hydroxyethyl methacrylate; HEMA-g-PTMeCar-Ind, 2-hydroxyethyl methacrylate-g-polylactide-carbonate-Indomethacin; HGH, human growth hormone; hMSC, human mesenchymal stem cells; Ind, indomethacin; LA, lactic acid; Met, metformin; MAA, methacrylic acid; MAP, mussel adhesive protein; MC, methyl cellulose; Met, metformin; MPEG-b-PDLLA, methoxy poly(ethylene glycol)-b-poly(lactic acid); MSC, mesenchymal stem cells; MVF, microvascular fragments; NIPAM, N-isopropylacrylamide; NIPAAm, poly(N-isopropylacrylamide); NMP, N-methyl-2-pyrrolidone; NS, normal saline; PBS, phosphate buffered saline; PCL, poly(ε-caprolactone); PCLA, poly(ε-caprolactone-co-lactide); PEI, polyethylenimine; PG, propylene glycol; PLA, poly(lactic acid-co-glycolic acid); PNIPAAm, poly(N-isopropylacrylamide); PTX, paclitaxel; PVA, poly(vinyl alcohol); QF, quetiapine fumarate; RGD, arginylglycylaspartic acid; SA, sodium alginate; SBF, simulated body fluid; SF, sodium fluorosilicate; STF, simulated tear fluid; TE, tissue engineering; TMZ, temozolomide; triMeCa, trimethylene carbonate; TSUN, 1,4,8-trioxa[4.6]spiro-9-undecanone; Van, vancomycin; VEGF, vascular endothelial growth factor.

a) This is the concentration used for the in vivo application, and it does not necessarily correspond to the critical gelation concentration of the system. When the system consists of a mixture of poloxamers only, the concentration given is the total concentration, while for mixtures with other additives the concentration stated is the one corresponding to the main thermoresponsive component. Note that any additives, such as polymers, drugs and cells, might affect the gelation properties.

b) The in vivo result is system dependent, i.e., the combination of gel matrix and active compound.
hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose, chitosan and Carbopol® (i.e. crosslinked poly(acrylic acid)) are well known for providing mucoadhesion. Similar to the previous studies, P407 or a combination of P407 and P188 were mixed with poly(vinyl alcohol), methylcellulose and its derivatives, such as carboxymethylcellulose and HPMC; sodium alginate, Carbopol®, chitosan, sodium poly(acrylate), glycerin, polycarbophil, methoxy poly(ethylene glycol)-b-poly(o,l-lactic acid) (poly(MPEG-b-PDLLA)) and glycosaminoglycans such as hyaluronic acid (HA). These systems were tested as delivery matrices for several compounds such as anticancer agents, anti-inflammatory/anti-fungal/anti-bacterial/anti-microbial drugs, cholesterol-lowering drugs, anti-diabetic drugs (insulin), anticonvulsant agents and vaccines. Similar observations as in the previous studies were made, such as biocompatibility, improved bioavailability of the compound, controlled and prolonged release, and control of tumour when anticancer agents were used. In one of the studies, the gel loaded with vitamins was injected in a model animal suffering from skin burn, and wound healing was observed due to the antioxidant action of the vitamins. In an interesting study by Luo et al, the combination of topical and controlled release of a compound from a TRG with photodynamic therapy was investigated. The gel matrix consisted of P407 and MPEG-b-PDLLA containing a two-photon absorption compound and a photosensitiser. The mixture was injected in tumour-bearing mice and then irradiated with near infrared (NIR) light to initiate photodynamic therapy. Prolonged retention of compounds on the tumour and inhibition of tumour growth were observed, with the compounds showing minimal cytotoxicity when non irradiated. In the only study on the TE concept, P407 was mixed with glycosaminoglycans and bone morphogenic protein (BMP-2), and upon injection in a model animal recovery of the injured cartilage was observed.

Another approach to modifying the properties of the poloxamers is to covalently link the polymer with other molecules or by further polymerising it to form pentablock terpolymers. The studies in which these approaches have been applied are discussed below.

In two of the studies, P407 was covalently linked to either chitosan or tetraaniline. In the case of chitosan-based polymer,
When polymerising in order to introduce fast degradability into the polymer structure, polymer scientists have copolymerised poly(ethylene oxide) (PEG) with ester-containing monomers, which degrade via hydrolysis; however, enzymatic degradation of ester bonds has been reported. Figure 5 shows the mechanism of hydrolysis of ester bonds, catalysed either via acid (top) or base (bottom). The most common units that are often combined with PEG to form degradable TRGs are ε-caprolactone (CL), ε-lactic acid (LA) and glycolic acid (GA), which are synthesised via ROP of the ester units on a PEG macroinitiator. Peptide-based degradable TRGs have also been applied in vivo, the degradation of which is enzymatically catalysed. When hydrolytically degradable polymers are concerned, it has been previously observed that the increase in the hydrophobicity of the polymer and the polymer concentration reduce the degradation rate, which is proportionally related to the drug release rate. As an example, the degradation rate decreases as the polymer is substituted from GA, to LA, to CL, thus allowing control of the degradation rate by modifying the polymer structure.

Diblock copolymers based on EG (A block) and an ester-containing unit (B block) were investigated. In two of the studies, the B block was based on either CL or both CL and P-dioxanone (DO). Upon injection of 20 wt% solution in rats, the gels were preserved for over a month, thus sustainably releasing bovine serum albumin (BSA) or bovine insulin. Compared to poloxamer 407, the gel of P407 was destabilised after 3 days, thus limiting the drug release efficacy. It was also observed that the presence of DO increased both the gelation and the release rate, which can be attributed to the increased hydrophilicity of DO compared to CL because of the extra oxygen in its structure. In the third study, the B block was based on LA and the 1,6-bis(p-carboxyphenoxo)hexane and in vivo biocompatibility was confirmed.

In several studies, ABA and BAB triblock copolymers with A and B blocks based on EG and CL, respectively, were investigated. Concentrated solutions of these copolymers (20–30 wt%) were injected in model animals, and in situ gelation and degradation of the gel were observed. Also, these thermogels provided sustained release of lidocaine, basic fibroblast growth factor or camptothecin (CPT)-containing particles. Interestingly, sustained and topical CPT release from the gel matrix both controlled the growth of tumour and prevented metastasis.

ABA and BAB triblock copolymers with the B block based on PLGA were also evaluated for in vivo gel formation and injectability. Both PEG-PLGA-PEG (PEG) and PLGA-b-PEG-b-PLGA (BAB) polymers have been patented by Macromed Inc. (acquired by Protherics UK Ltd) in 2000, and several articles have been published by Professor Sung Wan Kim (1940–2020, co-founder of Macromed Inc.) and other groups around the world since then. The patents cover both ABA and BAB polymers with total molar mass ranging from 2000 to 4990 g mol\(^{-1}\), hydrophobic content varying from 51 to 83 wt%, and a range of hydrophobic esters which are polymerised to form a distinct B block at various percentages. Amongst these, the best performing ones are BAB polymers with MM \(\approx 3100–4500\) g mol\(^{-1}\), hydrophobic PLGA content between 51 and 83 wt% and lactate content in the PLGA ranging from 65 to 85 mol%. The latter polymers are known as RegEl\(^{\text{TM}}\), and their gelation varies with the exact structural properties, i.e. MM and content in EG, LA and GA. Unlike Pluronics® which form traditional core–shell micelles above their critical micellisation temperature, RegEl\(^{\text{TM}}\) polymers form flower-like micelles, with the hydrophobic PLGA block forming the core of the micelle and the hydrophilic PEG block forming the ‘petals’ of the flower, also referred to as loops (Fig. 6). The gelation of these polymers relies on a polymer chain joining two micelles, as shown schematically in Fig. 6.

**Poloxamer 407 (w/v%)**

| 20 | 15 | 10 | 5 | 0 |
|----|----|----|----|----|
| 80 | 70 | 60 | 50 | 40 |

**Poloxamer 188 (w/v%)**

| 20 | 15 | 10 | 5 | 0 |
|----|----|----|----|----|
| 80 | 70 | 60 | 50 | 40 |

**Figure 4.** Gelation temperature \(T_{gel}\) as a function of the content in poloxamers 188 and 407. Data obtained from table 1 in Al Khateb et al. 2001

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Other degradable EG-based polymers besides poloxamer

Poloxamer’s structure is amphiphilic and consists of ether bonds, which are not hydrolytically degradable but they can be oxidised at a slow rate. In order to introduce fast degradability into the polymer structure, polymer scientists have copolymerised poly(ethylene glycol) (PEG) with ester-containing or carbamate-containing monomers, which degrade via hydrolysis; however, enzymatic degradation of ester bonds has been observed.

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It was concluded that the chemical bond between the polymers enhanced the stability of the gels compared to the gel formed by the physical mixture of P407 and chitosan. A solution of P407-g-chitosan was loaded with poly(lactic acid-co-glycolic acid) (PLGA) nanoparticles containing vaccines, and upon in vivo injection, successful vaccine delivery was observed. In another study, P407 was covalently linked to tetraaniline, which is electroactive. It was observed that the presence of tetraaniline enhanced the strength of the gel, and in vivo gelation was confirmed.

Ring opening polymerisation (ROP) of poloxamers facilitates the synthesis of pentablock copolymers. When polymerising trimethylene carbonate (triMeCa) on P407, it was observed that the pentablock terpolymer showed enhanced gelation and improved cytotoxicity compared to P407. Injection of 5 wt% copolymer solution containing mitomycin C in rabbits, which were previously undergone a glaucoma filtration surgery, improved their health. In two studies by Li et al., l-lactic acid formed the outer blocks of a pentablock terpolymer based on P105. In both studies, the gel matrix, formed by concentrated solutions (35–40 wt%), was tested as a drug delivery depot for either antimicrobial peptides or anticancer agents, such as docetaxel (DTX) and oxaliplatin. Local and controlled release of the compound was observed upon injection, while in the case of delivery of anticancer agents growth of tumour, angiogenesis and tumour cell proliferation were suppressed, which subsequently prevented metastasis. However, it should be noted that the concentrations used in the last two studies are relatively high; thus their commercial application might be limited because of high cost.

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Concerning the ABA polymers, in vivo gelation, biocompatibility and degradation were confirmed.\textsuperscript{117–119} Similarly to their ABA counterparts, the BAB triblock copolymers are biocompatible\textsuperscript{122} and gel and degrade in vivo.\textsuperscript{30,122} Their concentrated solutions (20–25 wt%) were also tested in vivo as drug delivery systems.\textsuperscript{30,31,121–127} The TRGs successfully served as matrices for sustained release of (i) interleukin-2, thus inhibiting growth of tumour,\textsuperscript{126,127} (ii) huperzine A-phospholipid, thus reducing its toxic side effects,\textsuperscript{121} (iii) Avastin®, which increased the half-life of the drug,\textsuperscript{30} (iv) 5-fluorouracil, which prevented adhesion in the Achilles tendon, important to avoid pain after surgery,\textsuperscript{122} (v) dexamethasone acetate for improved ocular drug delivery,\textsuperscript{120} (vi) insulin or glucagon-like peptide for the treatment of diabetes\textsuperscript{123–125} and (vii) irinotecan, and tumour regression was observed.\textsuperscript{31}

In three studies, end-capped PCLA-b-PEG-b-PCLA triblock copolymers were investigated, where PCLA stands for poly(\(\varepsilon\)-caprolactone-co-lactide),\textsuperscript{32,128,129} Acetyl,\textsuperscript{42,129} 2-(2',3',5'-triiodobenzoyl) (TIB),\textsuperscript{42,129} and propionyl\textsuperscript{128} groups were used as end-capping units. TIB groups were introduced into the structure to allow visualisation, but this caused solubility issues due to the increased hydrophobicity.\textsuperscript{42} 25 wt\% copolymer solution with an acetyl/TIB ratio of 25/75 was in vivo administered while in the gel phase (\(T_{\text{gel}}\) at 10–15 °C), and in vivo degradation was confirmed,\textsuperscript{42} while the acetyl analogue promoted in vivo sustained release of celecoxib (COX).\textsuperscript{129} The propionyl-capped polymer solutions were investigated as effective COX carriers in mice and dogs.\textsuperscript{128} Biocompatibility was confirmed after injection in mice, while intradiscal injection in dogs suffering from intervertebral disc degeneration improved their health.\textsuperscript{128}

BAB triblock copolymers based on PEG (A block) and on a random copolymer of CL with either GA\textsuperscript{130} or 1,4,8-trioxa[4.6]spiro-9-undecanone (TSUN)\textsuperscript{131} (B block) were also applied in vivo.\textsuperscript{25} wt% GA-based copolymer solution containing liraglutide was injected in mice and in vivo biocompatibility, degradation and hypoglycaemic efficacy were confirmed (Fig. 7).\textsuperscript{130} A three-component system consisting of 25 wt% TSUN-based copolymer solution, doxorubicin (DOX) and iodine\textsuperscript{131}-labelled HA was injected in tumour-bearing mice, which provided local administration of DOX and inhibition of tumour growth.\textsuperscript{131}

Degradable linear multiblock copolymers based on PEG were also tested as in vivo thermogels.\textsuperscript{29,157} In one of the studies, a
17 wt% solution of PEG-b-PCL-b-PLA-b-PCL-b-PEG, loaded with PLGA nanoparticles and vaccines,\textsuperscript{29} was studied; PCL and PLA stand for poly(\(\varepsilon\)-caprolactone) and poly(lactic acid), respectively. The second study investigated 25 wt% solutions of multiblock copolymers based on PEG, poly(propylene glycol) and poly(polytetrahydrofuran carbonate), loaded with DOX. The TRGs were injected in tumour-bearing mice, and successful inhibition of tumour growth was observed.\textsuperscript{29,137}

An interesting systematic study in which four-star copolymers were investigated as in vivo gelators was also reported.\textsuperscript{138} The

![Figure 6](image1)

**Figure 6.** Regen polymers PLGA-b-PEG-b-PLGA, where PLGA and PEG stand for poly(lactic-co-glycolic acid) and poly(ethylene glycol) respectively, adopt a flower-like micelle configuration in aqueous media. When the critical gelation concentration and temperature, CGC and CGT, respectively, are reached, gelation occurs via hydrophilic bridges by unfolded polymer chains.

![Figure 7](image2)

**Figure 7.** (a) In vivo degradation of the BAB triblock copolymer with B consisting of caprolactone (CL) and glycolic acid (GA) and the A block being based on ethylene glycol (EG). (b) In vivo release of liraglutide from the hydrogel. (c) Blood glucose levels monitored over time; pink, blue and red represent the results after injection of the blank hydrogel, daily injection of the solution of the drug only, and a single injection of the drug trapped within the gel matrix, respectively. Adopted with permission from Chen Y, Luan J, Shen W, Lei K, Yu L and Ding J, ACS Appl Mater Interfaces 8:30703–30713 (2016), Copyright (2017) American Chemical Society.\textsuperscript{130}
core of the star was based on PLGA of various LA/GA molar ratios, whereas the arms were based on methoxy PEG (MPEG). 30 wt% of the copolymer solutions were injected in rats and rapid gelation, degradation and good biocompatibility were confirmed.138

Incorporation of carbamate bonds also introduces degradability into the system. Two studies, carried out by Wang’s group, were based on ABA triblock copolymers with A and B blocks based on EG and serinol hexamethylene urethane.132,133 In vivo biocompatibility and controlled release of bevacizumab were observed.132,133

All the studies discussed so far are based on hydrolytically degradable systems, the degradation of which can be accelerated in an acidic environment. Chen’s group investigated polymers which are based on polypeptides and PEG, and thus their degradation is enzymatically catalysed.134–136 In two of the studies, L-alanine and L-phenylalanine composed the peptide part.135,136 8 wt% of polypeptide solution was injected in rats and in vivo biocompatibility and degradation were confirmed.136 In vivo injection of the polymer solution containing combrestatin A-4 particles and DTX showed inhibition of tumour growth.136 When mixed with bone marrow mesenchymal stem cells and injected in rabbits with osteochondral defect, cartilage regeneration was observed.135 In an interesting study by the group, L-methionine was used instead to protect the cells from reactive oxygen species.134 Upon exposure to hydrogen peroxide (H₂O₂), the thioether group of L-methionine is oxidised to sulfoxide and sulfone groups. Thus, the polymer becomes more hydrophilic, leading to destabilisation and degradation of the gel. Upon injection in rats, in vivo degradation was completed after 6 weeks.134

**PNIPAAm-based TRGs**

PNIPAAm is one of the most popular thermoresponsive polymers (Fig. 2) and it has been extensively investigated either as a homopolymer or by co-polymerising its repeated unit, i.e. N-isopropylacrylamide (NIPAAm), with other units in order to obtain the desired properties.4 Below its LCST, NIPAAm units interact with water via hydrogen bonding; however, upon increasing the temperature, hydrogen bonding between the different NIPAAm units is favoured instead, thus leading to a thermoresponsive (Fig. 8). As its cloud point (CP) is around 31 °C and independent of the MM and composition,206 it has attracted much interest for biomedical applications. Due to its promising properties, in vivo applications of NIPAAm-based random,139–142,144,146,148 block,146–149 conjugate163,164 and star166 synthetic copolymers as well as NIPAAm-based polymers covalently183,184,195,196 or physically mixed143,145,151,152 with natural components were reported in the literature.

Random NIPAAm-based copolymers, synthesised via free radical polymerisation, were applied in model animals as TRGs.139–142,144 Several units have been used as comonomers such as acrylic acid (AA),141,142 γ-butyrolactone acrylate,142 N-hydroxysuccinimide,143 acryloyloxy dimethyl-γ-butyrolactone,144 poly(ethylene glycol) methyl ether methacrylate,140 methacrylic acid140,144 and n-butyl acrylate (BuA).139 Upon injection of the solutions in model animals, in situ gelation139–142 and in vivo biocompatibility were confirmed.139–142 Interestingly, in one of the studies, tetraaniline was incorporated into the structure to provide the electroactive and antioxidant properties needed for myocardial infarction therapy.140 In another study, a macromonomer on which the drug was covalently linked, namely 2-hydroxyethylmethacrylate-g-poly(trimethylene carbonate)-indomethacin, was copolymerised with NIPAAm, and anti-inflammatory action for treatment of uveitis was confirmed.144

In an interesting study by Bayat et al., the TRG was in vivo investigated as a possible sealant in ocular trauma.139 The solution was injected using an in-house developed syringe, and practical workshops in which this copolymer solution was applied ex vivo in pig eyes were held. Military opthalmologists and clinicians participated in the workshop, and they were briefly instructed on how to apply the injectable gel. On the first attempt, 43% of the participants achieved the application of the TRG, and all of them were successful on the second time.139 This highlights the applicability of TRGs as injectable gels.

(A-co-C)–b–b–(A-co-C) triblock copolymer, where A, B and C units are based on NIPAAm, EG and BuA, respectively, was applied as wound dressing.1 The copolymer solution was mixed with silver nanoparticle decorated reduced graphene oxide (GO) nanosheets, and the mixture was applied as a spray on the skin, after which gelation was observed.1 In two studies by Gupta et al., ABC triblock copolymers were synthesised via reversible addition-fragmentation chain transfer polymerisation and were tested as in vivo drug release depots.146,147 In both studies, B and C blocks were based on N,N-dimethylacrylamide and NIPAAm, respectively.146,147 The A block was based on either propylene sulfide (PSu)146,147 or CL147 and PLGA.147 The A block was synthesised via ROP and was incorporated into the structure to provide degradation via different mechanisms, thus controlling the drug release at a different rate: PSu provides reactive oxygen species degradation,146,147 while PCL and PLGA provide slow and fast hydrolysis/enzymatic degradation, respectively.147 Interestingly, in one of the studies, the copolymers formed gels at a low concentration of 2.5 wt%.146 5 wt% of the copolymer solutions were loaded with Nile red and were injected in mice.146,147 Sustained release from the triblock copolymer was observed over 14 days, while burst release was observed when the corresponding AB diblock copolymer was used as a control (Fig. 9).146 In vivo degradation was controlled by the degradation mechanism and thus the PLGA-based polymer released 90% of the stain in 2 days, whereas the other two copolymers sustained the release for 12 days.147

As the progress in polymer science facilitates the synthesis of more complicated architectures, ABCBA pentablock terpolymers with A, B and C being based on NIPAAm, CL and EG, respectively, have also been applied in vivo.148 These polymers were synthesised via atom transfer radical polymerisation (ATRP) and ROP, and in situ gel formation was confirmed upon injection of 20 wt % polymer solution in rats.148

Branch copolymers based on poly(PEG-co-NIPAAm), where PEG stands for poly(ethylene glycol) citrate, were synthesised via a combination of poly(condensation) reaction and free-radical
polymerisation. Incorporation of citrate groups in the structure gives antioxidant properties to the final copolymer. In vivo studies confirmed biocompatibility, degradability, controlled release of chemokine and new tissue formation. Interestingly, these copolymers were mixed with gelatin and either multipotent adipose-derived cells, or immortalised mouse embryonic fibroblasts and upon injection osteogenic differentiation and vascularisation were observed. When mixed with mesenchymal stem cells (MSC) and either GO or strontium ions, in vivo osteoinduction, mineralisation, osteoinduction, angiogenesis and bone formation were observed. Nevertheless, one should bear in mind that these copolymers possess a broad molar mass distribution (MMD, final D of 3.41), and thus they consist of polymer chains with different chemical structures and different properties. These copolymers are also difficult to be reproduced, and therefore controlled synthetic techniques should be used for the synthesis of well-defined copolymers (MMD closer to 1).

Conjugate and star copolymers have also been applied in vivo. In the studies on conjugate polymers, poly(NIPAAm) was linked with poly(serinol hexamethylene urea) for the delivery of vascular endothelial growth factor for the treatment of myocardial infarction. It is worth noting that these copolymers formed a gel at concentrations as low as 1 wt% and upon in vivo injection vascularisation and the formation of vascular endothelial cells were observed. Star copolymers based on NIPAAm, AA, O-phosphoethanolamine, synthesised via ATRP, formed stable gels at only 0.5 wt% and in vivo mineralisation of the hydrogel was promoted.

The combination of poly(NIPAAm) with natural components such as HA, gelatin, chitosan, dextran, mussel adhesive protein (MAP) and heparin was also found in the literature. These naturally based polymers were either mixed with NIPAAm homopolymer or covalently linked.

Two studies on either a physical mixture or conjugate polymer were reported in the literature as drug delivery depots. In the first study, poly(NIPAAm) was mixed with conjugated polymer of dextran, CL and 2-hydroxyethyl methacrylate, while in the second study heparin was conjugated with poly(NIPAAm). In vivo controlled release of BSA and ibuprofen was observed, and the anti-inflammatory action of ibuprofen and consequent wound healing were confirmed.

In several studies, poly(NIPAAm) was combined with HA, and in vivo gelation was observed. When a graft copolymer was synthesised, it was investigated as a drug delivery matrix by loading with either BSA, bioactive microvascular fragments or gentamicin. The controlled drug release was shown to improve vascularisation and prevent bacterial colonisation. When injected in a rabbit with osteochondral defect, cartilage formation was observed, thus replacing the gel matrix 12 weeks post-injection. In another study, HA methacrylate was copolymerised with NIPAAm and the random copolymer was compared to the physical mixture of HA and NIPAAm. Both the copolymer and the physical mixture were loaded with adipose derived stem cells, and they were intra-articularly injected in rabbits. It was observed that cartilage formation was enhanced when the copolymer was used instead of the mixture.

Grafting of NIPAAm on gelatin and chitosan and MAP was also reported, primarily in the concept of TE. In two of the studies, a solution of either NIPAAm-g-gelatin or NIPAAm-g-chitosan, mixed with MSC, was injected in model animals, and in situ gelation and enhanced tissue formation were observed. To combine the promising effects of both gelatin and chitosan, PNIPAAm was grafted on both natural polymers, and upon addition of biphasic calcium phosphate and subsequent in vivo injection the formation of bone tissue was observed. For adipose TE, PNIPAAm was linked on MAP and decellularised adipose tissue was added in the solution, thus promoting in vivo angiogenesis. In the concept of drug delivery, a NIPAAm-g-gelatin solution was loaded with pilocarpine, and controlled and topical drug release improved tissue damage in glaucomatous eyes.

**Poly(organophosphazene)-based TRGs**

A different class of injectable thermogels which were tested in vivo is poly(organophosphazene)s (Fig. 2 with R representing an organic group). These studies were published by Song’s group and their collaborators. In the studies, the backbone was functionalised with different groups, such as the hydrophobic isoleucine ethyl ester, a hydrophilic EG-based group, the hydrolysable glycolic lactate ethyl ester, and di(glycine) and di(glycine). Poly(organophosphazene)s were tested as drug delivery matrices for DOX, PTX and DTX. Upon injection in tumour-bearing mice, local administration and control of tumour growth were observed. When the same thermogel was injected, faster release of PTX was detected compared to DOX, confirming the different pharmacokinetics and indicating the necessity of investigating the drug release on a defined application. In the afore-mentioned studies, the polymer was physically mixed with the drug. However, studies in which the drug, e.g. DOX, camptothecin or PTX, was covalently linked to the polymer were also reported. The solution was injected in tumour-bearing mice and the drug was locally released in a prolonged period via hydrolytic degradation, thus effectively inhibiting the tumour growth and reducing the side effects. Poly(organophosphazene)s were also used as gel depots for delivering human growth hormone (HGH) and BMP-2. In
the case of HGH, the polymer was mixed with poly-L-arginine or modified with protamine to form complexes with HGH via electrostatic attraction, while in the case of BMP-2 the complexes were formed via both hydrophobic and electrostatic attractions. Upon injection in different model animals, controlled release was observed. Interestingly, in the case of BMP-2, bone formation was observed. In a similar concept, cell proliferation was observed when a mixture of polyphosphazene, collagen and pre-osteoblasts was injected in mice.

In two of the latest studies of the group, polyethyleneimine (PEI) was covalently linked to the polymer to promote complex formation for gene delivery. In the first study, controlled co-delivery of gene and drug was confirmed for up to a month. Interestingly, a combination of photothermal and genetic combination therapy was attempted by fabricating a triple target system. The polymer was chemically modified with (i) PEI, which electrostatically interacts with siRNA, thus protecting it from endosomal and enzymatic degradation, and (ii) folate groups, which target the folate receptor, overexpressed in cancer cells. Au-Fe$_3$O$_4$ particles were also incorporated in the system for magnetic targeting and NIR-induced hyperthermia. Thus, the targeted therapy and a reduction in side effects were achieved via (i) an enhanced permeability and retention effect, (ii) magnetism, (iii) folate recognition and (iv) NIR-induced hyperthermia.

**PETOX-based TRGs**

Poly(2-oxazoline)s are a special class of polymers that mimic the structure of the proteins, and thus have received much attention since their discovery in the late 1960s. These polymers, synthesised via cationic ROP, consist of an amide group with the nitrogen and the carbonyl groups being part of the polymer backbone and side chain, respectively. The general structure is shown in Fig. 2 with R representing the side group, which is varied to modulate the final properties of the polymers, such as hydrophilicity/hydrophobicity. Amongst the different poly(2-oxazoline)s, PETOX is thermoresponsive and shows great promise as it is FDA approved to be used as an indirect food additive, and a PETOX-based formulation has reached clinical trials (Phase 1).

A few studies on the in vivo application of TRGs containing PETOX have been reported in the last decade. In two of the studies, PETOX-b-PCL-b-PETOX triblock copolymers were injected in the eyes of model animals, and biocompatibility was confirmed. Interestingly, the oxazoline-based copolymer showed superior biocompatibility compared to Pluronic F127 and Matrigel, a natural protein mixture showing thermoreversible gelation. In the third study, PETOX-b-PLGA-b-PETOX polymers were injected in mice, and in vivo biodegradation and controlled release of salmon calcitonin were confirmed, the latter successfully regulating the level of calcium in blood.

**CLINICAL APPLICATIONS**

Numerous studies have been published on the in vivo applications of TRGs in model animals, with mice and rabbits being amongst the most common. However, to the best of our knowledge, only two TRGs have reached clinical trials, Pluronic and Regel, showing the increased number of requirements that need to be satisfied prior to clinical studies and commercialisation. Their applications in model animals have been discussed in the relevant sections. When PTX, an anticancer agent, is incorporated in the ReGel solution, the system is known as OncoGel, and it has been applied in clinical trials (NCT00479765, NCT00573131). However, these clinical trials have been terminated, due to sponsor decision (NCT00479765) or because the product did not show any impact on tumour regression (NCT00573131). On the other hand, several trials of Pluronic F127 (poloxamer 407) have been completed and/or are ongoing. Pluronic F127 has been tested as a drug delivery matrix of simvastatin (NCT03400475) and antralin (NCT03348462) for the treatment of mastitis and psoriasis, respectively. Its formulation with Pluronic F68 (poloxamer 188) has been applied in humans for the delivery of metronidazole (NCT02365389) for treating bacterial vaginosis, while a formulation containing P407 and P188 is currently under investigation for the delivery of timolol (NCT04139018) for the treatment of epistaxis. Nevertheless, one should bear in mind that these formulations are in the gel state below room temperature; thus application/injection is undertaken whilst the sample is in the gel state.

**CONCLUSIONS**

In summary, a plethora of TRGs have been studied in model animals, and several drugs have been incorporated into the gel matrix attempting to treat many different diseases like cancer, osteoporosis and diabetes. These systems, when tested as drug delivery depots, provide controlled and local drug release, thus avoiding undesired side effects, while in TE tissue formation was observed. Despite the fact that poloxamers are the most studied type of thermoresponsive polymers, they present in vivo instability; thus attempts to improve their gelation properties have been made by mixing of poloxamers and/or other additives. As their gelation temperature reduces below r.t. when the concentration increases from 15 w/w% to 20 w/w%, in some cases poloxamers are preferable for treating skin disorders, in which epicutaneous application is required. A great deal of research has been focused on the in vivo application of ester- or carbamate-containing PEG-based polymers, owing to their degradability. Interesting studies based on PNIPAAm have also been carried out, but some of the syntheses were carried out using non-controlled polymerisation techniques which might limit the reproducibility of the results. A limited number of studies on poly(organophosphazene)s and poly(2-oxazoline)s have been found in the literature and have been discussed. Finally, to the best of our knowledge, only the OncoGel system (Regel with PTX) and poloxamer 407 (in some cases in formulation with poloxamer 188) have successfully reached clinical applications, indicating the difficulty of achieving the appropriate combination of structural parameters in order to obtain the desired properties for in vivo application. Nevertheless, TRGs remain very promising, due to their ease in application. Therefore, designing the TRG that meets the desired properties for the final application is highly important in the health sector, in which the TRG system will provide local and sustained drug delivery, thus minimising undesired side effects, or promote tissue regeneration.

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