



Review article

In situ-forming hydrogels—review of temperature-sensitive systems

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Abstract

In the past few years, an increasing number of in situ-forming systems have been reported in the literature for various biomedical applications, including drug delivery, cell encapsulation, and tissue repair. There are several possible mechanisms that lead to in situ gel formation: solvent exchange, UV-irradiation, ionic cross-linkage, pH change, and temperature modulation. The thermosensitive approach can be advantageous for particular applications as it does not require organic solvents, co-polymerization agents, or an externally applied trigger for gelation. In the last 2 decades, several thermosensitive formulations have been proposed. This manuscript focuses on aqueous polymeric solutions that form implants in situ in response to temperature change, generally from ambient to body temperature. It mainly reviews the characterization and use of polysaccharides, *N*-isopropylacrylamide copolymers, poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (poloxamer) and its copolymers, poly(ethylene oxide)/(D,L-lactic acid-*co*-glycolic acid) copolymers, and thermosensitive liposome-based systems.

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1. Introduction

In the past few years, an increasing number of in situ-forming systems have been reported in the literature for various biomedical applications, including drug delivery, cell encapsulation, and tissue repair. These systems are injectable fluids that can be introduced into the body in a minimally invasive manner prior to solidifying or gelling within the desired tissue, organ, or body cavity. Injectable gel-forming matrices offer several advantages over systems shaped into their final form before implantation. For example, injectable materials do not require a surgical procedure for placement (and withdrawal if not biodegradable), and various therapeutic agents can be incorporated by simple mixing. When they are used to fill a cavity or a defect, their flowing nature enables a good fit. In situ implant formation can occur as a result of either a physical or chemical change of the system.

There are several possible mechanisms leading to in situ implant formation. The solvent exchange approach consists of dissolving a water-insoluble polymer in a water-miscible, biocompatible solvent. Upon contact with body fluids, the solvent diffuses out of the polymer while water permeates the liquid polymer matrix. Due to its insolubility in water, the polymer precipitates, resulting in the formation of a solid polymeric implant [1–5]. However, incomplete implant formation can be observed in vivo resulting in a high initial release and local or systemic toxicity. Also, the organic solvent used to solubilize the polymer can physically denature labile compounds such as proteins. Photopolymerization has also been proposed to prepare in situ implants. This approach has been taken to produce depot formulations [6], biological adhesives for soft tissues [7–10], and orthopaedic biomaterials [11]. However, photopolymerization requires the presence of a photoinitiator at the gelation site, which can be toxic. Furthermore, the penetration capacity of the radiation source limits the number of application sites, and the reaction can evoke enough heat to damage surrounding tissues.

In situ-forming systems that do not require organic solvents or copolymerization agents have gained increasing attention. These are liquid aqueous solutions before administration, but gel under physiological conditions.

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Gelation can occur in situ by ionic cross-linking [12,13] or after a change in pH [14,15] or temperature. The latter approach exploits temperature-induced phase transition.

Some polymers undergo abrupt changes in solubility in response to increases in environmental temperature (lower critical solution temperature, LCST). This phase separation is generally viewed as a phenomenon governed by the balance of hydrophilic and hydrophobic moieties on the polymer chain and the free energy of mixing [16–18]. The free energy of association varies with enthalpy, entropy and temperature ($\Delta G = \Delta H - T\Delta S$). As the positive enthalpy term (ΔH) is smaller than the entropy term (ΔS), an increase in temperature results in a larger $T\Delta S$, making ΔG negative and favoring polymer chain association. The temperature dependence of certain molecular interactions, such as hydrogen bonds and hydrophobic effects, contribute to phase separation. At the LCST, hydrogen bonding between the polymer and water becomes unfavorable, compared to polymer–polymer and water–water interactions, and an abrupt transition occurs as the solvated macromolecule quickly dehydrates and changes to a more hydrophobic structure [18,19]. Alternatively, some amphiphilic polymers, that self-assemble in solution, show micelle packing and gel formation because of polymer–polymer interactions when temperature is increased [20].

The ideal system would be a solution that is a free-flowing, injectable liquid at ambient temperature. It should then gel at body temperature with minimal syneresis. Moreover, loading with drugs or cells should be achieved by simple mixing. When administered parenterally, these systems should exhibit a pH close to neutrality and should be bioresorbable. This paper focuses on polymeric solutions that can form implants in situ in response to temperature change, from ambient to body temperature. It mainly reviews the characterization and use of polysaccharides, *N*-isopropylacrylamide (NIPAM) copolymers, poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (PEO-PPO-PEO) and its copolymers, poly(ethylene oxide)/(D,L-lactic acid-*co*-glycolic acid) (PEO/PLGA) copolymers, and thermosensitive liposome-based systems (Fig. 1).

2. Polysaccharides

2.1. Cellulose derivatives

Thermoreversible gels can be prepared with naturally occurring polymers. Most natural polymer aqueous solutions form a gel phase when their temperature is lowered. Classic examples of natural polymers exhibiting a sol–gel transition include gelatin and carrageenan. At elevated temperatures, these polymers adopt a random coil conformation in solution. Upon cooling, a continuous network is formed by partial helix formation [21,22]. Some cellulose derivatives are an exception to this gelation mechanism. At low concentrations (1–10 wt%), their aqueous solutions are

liquid at low temperature, but gel upon heating. Methylcellulose (Fig. 1A) [23,24] and hydroxypropyl methylcellulose (HPMC) (Fig. 1B) [24] are typical examples of such polymers. Methylcellulose solutions transform into opaque gels between 40 and 50 °C [23,24], whereas HPMC shows phase transition between 75 and 90 °C [24]. These phase transition temperatures can be lowered by chemical or physical modifications. For example, NaCl decreases the transition temperature of methylcellulose solutions to 32–34 °C [23,24]. Similarly, by reducing the hydroxypropyl molar substitution of HPMC, its transition temperature can be lowered to ~40 °C [24].

Gelation of methylcellulose or HPMC solutions is primarily caused by the hydrophobic interaction between molecules containing methoxy substitution. At low temperatures, the macromolecules are hydrated, and there is little polymer–polymer interaction other than simple entanglement. As the temperature is raised, the polymers gradually lose their water of hydration, which is reflected by a decline in relative viscosity. Eventually, when sufficient but not complete dehydration of the polymer occurs, polymer–polymer associations take place, and the system approaches an infinite network structure, as reflected experimentally by a sharp rise in relative viscosity [23].

This sol–gel transformation has been exploited to design in situ gelling systems. Tate et al. [25] evaluated methylcellulose-based constructs as potential tissue engineering scaffolds for the repair of brain defects. These systems exhibited low viscosity at 23 °C and formed soft gels intracerebrally at 37 °C. The gels were biocompatible both in the presence of cultured cells and in the injured rat brain.

Aqueous solutions of ethyl(hydroxyethyl)cellulose (EHEC) (Fig. 1C) also exhibit thermosensitive behavior. However, their viscosity decreases with temperature, which is not appropriate for the preparation of in situ-forming implants. At the end of the 1980s, Carlsson et al. [26,27] reported that the addition of an ionic surfactant, like sodium dodecyl sulphate or cetyl triammonium bromide, to semi-dilute (1–4 wt%) EHEC solutions completely changed their thermal behavior. These systems underwent sol–gel phase transition upon heating from room temperature to 30–40 °C, resulting in the formation of stiff and clear gels. The rheological properties of such gels were further investigated by Nyström et al. [28]. The surfactant was found to interact with EHEC by a strongly cooperative process implying the formation of micelle-like surfactant clusters on the polymer [27,29,30]. Binding increased with rising temperature, and gelation was attributed to the ability of micelle-like clusters on the polymer chain to couple with segments on other chains [27,29].

Scherlund et al. [31] evaluated the EHEC/surfactant system for the local delivery of anesthetic agents to the periodontal pocket. They incorporated small amounts of lidocaine and prilocaine into the solution without affecting gelation behavior. The tested formulations showed

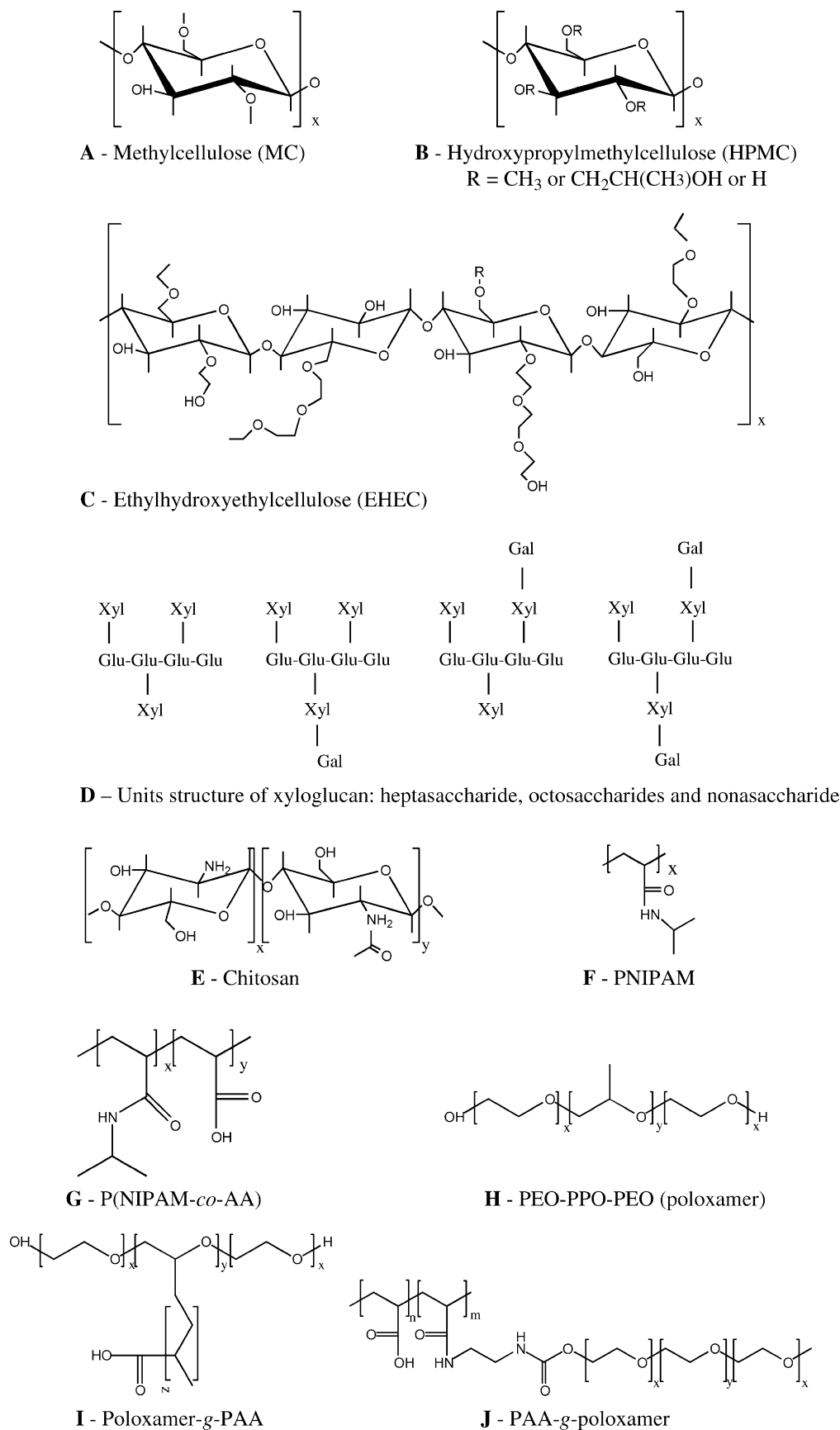
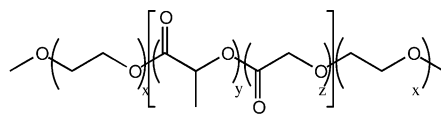
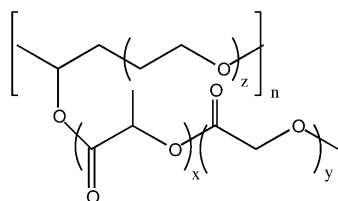
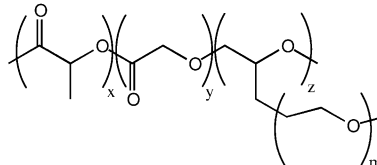
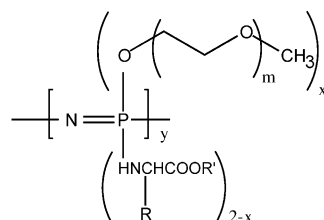


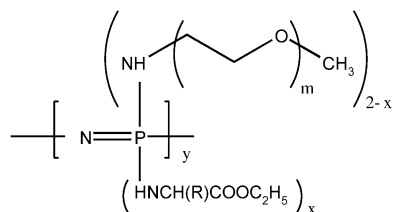
Fig. 1. Chemical formulas of hydrogel-forming polymers.

**K** - PEO-PLGA-PEO**L** - PEO-g-PLGA**M** - PLGA-g-PEO

R = H (Gly), CH₃ (Ala), COOR' (Mal), CH₂COOR' (Asp), CH₂CH₂COOR' (Glu)

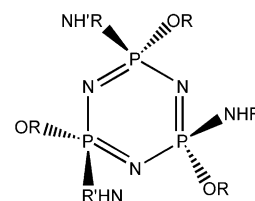
R' = CH₃ (Me), C₂H₅ (Et), CH₂C₆H₅ (Bz)

N - Poly(organophosphazene) derivatives



R = CH(CH₃)CH₂CH₃

O - Poly(organophosphazene) derivatives



OR = alkoxy PEG

NHR' = amino acid esters

P - Cyclotriphosphazenes

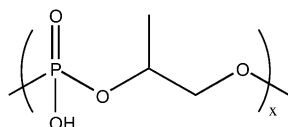
**Q** - Poly(1,2-propylene phosphate)

Fig. 1 (continued)

sustained drug release over a minimum of 60 min, making them interesting for short-term pain control. From a toxicological point of view, the need for inclusion of an ionic surfactant in such a formulation may, however, impair its clinical development.

2.2. Xyloglucan

Xyloglucan (Fig. 1D), a polysaccharide derived from tamarind seed, forms thermoresponsive gels in water, under certain conditions. Xyloglucan is composed

of a (1-4)- β -D-glucan backbone chain (GLU) which presents (1-6)- α -D-xylose branches (XYL) partially substituted by (1-2)- β -D-galactoxylose (GAL). Tamarind seed xyloglucan is composed of three units of xyloglucan oligomers with heptasaccharide, octosaccharide and nanosaccharide, which differ in the number of galactose side chains. When xyloglucan is partially degraded by β -galactosidase, the resultant product exhibits thermally reversible gelation in dilute aqueous solutions. Such behavior does not occur with native xyloglucan. Gelation is only possible when the galactose removal ratio exceeds $\sim 35\%$ [32]. The transition temperature is inversely related to polymer concentration [33] and the galactose removal ratio [32]. For example, the sol–gel transition of xyloglucan was shown to decrease from 40 to 5 °C when the galactose removal ratio increased from 35 to 58%.

Xyloglucan gels have been evaluated for the rectal delivery of indomethacin in rabbits [33]. They provided a broader absorption peak and longer residence time than commercial suppositories. Moreover, morphological studies of rectal mucosa after a single administration showed no evidence of tissue damage. Intraperitoneal administration of mitomycin C in a 1.5-wt% xyloglucan gel to rats resulted in a broad concentration–time profile, as opposed to a narrow peak and rapid disappearance from the peritoneal fluid and plasma when the drug was given as a solution [34]. In two other studies, the gels were investigated as vehicles for the oral delivery of indomethacin [35] and theophylline [36]. The bioavailability of indomethacin from xyloglucan gels was increased approximately three-fold compared to the control suspension. Likewise, theophylline bioavailability was 1.7–2.5 times higher than that of the commercially available oral, sustained-release liquid dosage form. Xyloglucan formulations were also assessed for ocular delivery of pilocarpine, using poloxamer 407 as a positive thermo-sensitive control [37]. The 1.5 wt% xyloglucan formulation enhanced the miotic response to a degree similar to that of a 25 wt% poloxamer 407 gel. More recently, xyloglucan gels were evaluated for the percutaneous administration of non-steroidal anti-inflammatory drugs [38]. After topical application, the xyloglucan formulations performed better than poloxamer 407 gels in improving the bioavailabilities of ibuprofen and ketoprofen. As for cellulose derivatives, xyloglucan solutions gel at low concentrations (1–2 wt%), and this may be advantageous from a toxicological viewpoint as the amount of administered polymer is low. In addition, xyloglucan is approved for use as a food additive. However, its relatively low transition temperature (22–27 °C) makes handling at room temperature problematic.

2.3. Chitosan and glycerophosphate

Chenite et al. [39,40] developed a novel approach to produce thermally sensitive neutral solutions based on

chitosan/polyol salt combinations. Chitosan (Fig. 1E) is the deacetylated derivative of chitin, a natural component of shrimp and crab shells. It is a biocompatible, pH-dependent cationic polymer, which is soluble in water up to pH 6.2. Basification of chitosan aqueous solutions above this pH leads to the formation of an hydrated gel-like precipitate. Phase separation ensues from the neutralization of chitosan amine groups and the consequent elimination of repulsive interchain electrostatic forces, which subsequently allow for extensive hydrogen bonding and hydrophobic interactions between chains. pH-gelling, cationic chitosan solutions have been transformed into thermally sensitive, pH-dependent, gel-forming systems by the addition of polyol salts (e.g. β -glycerophosphate, GP). These formulations possess a neutral pH, remain liquid at or below room temperature, and form monolithic gels at body temperature. The stability of the sol at room temperature and the gelation time increase as the chitosan degree of deacetylation decreases [41]. Like other polysaccharide systems, the gels are obtained at low polymer concentrations (~ 2 wt%).

Solubility at low temperatures is probably due to hydration of the chitosan promoted by GP. Upon heating, the chitosan chains lose their water of hydration, bonding between chains can occur and gelation proceeds. Three types of interactions may be involved in the gelation process: (1) electrostatic attraction between the ammonium groups of chitosan and the phosphate group of GP; (2) hydrogen bonding between polymer chains as a consequence of reduced electrostatic repulsion after neutralization of the chitosan solution with GP; and (3) chitosan–chitosan hydrophobic interactions. Readers are referred to the original papers for more details on the gelation mechanism of chitosan/GP solutions [40,41].

In parallel, our group evaluated the chitosan/GP system for pharmaceutical applications [41–43]. Stability studies showed that solutions made with 84% deacetylated chitosan could be stored for 3 months at 4 °C without apparent change in viscosity. In vitro release experiments revealed that the system could deliver macromolecules (M_w 12,000–148,000) over a period of several hours to a few days [41]. However, due to high gel porosity, low molecular weight hydrophilic compounds (< 1000 g/mol) were completely released within hours. To achieve sustained delivery that would be independent of the drug's molecular weight, hydrophilic compounds were first loaded into liposomes, which were then mixed with the thermosensitive solution [42]. This approach substantially slowed the release rate (Fig. 2), and the release profile of the incorporated compound could be controlled by adjusting liposome characteristics, such as size and composition. Recently, we proposed to use the thermosensitive hydrogel for the local administration of paclitaxel, a hydrophobic anticancer drug, at tumor resection sites to prevent local tumor recurrence [43]. In vitro release profiles demonstrated controlled drug delivery for over 1 month. In mice, intratumoral

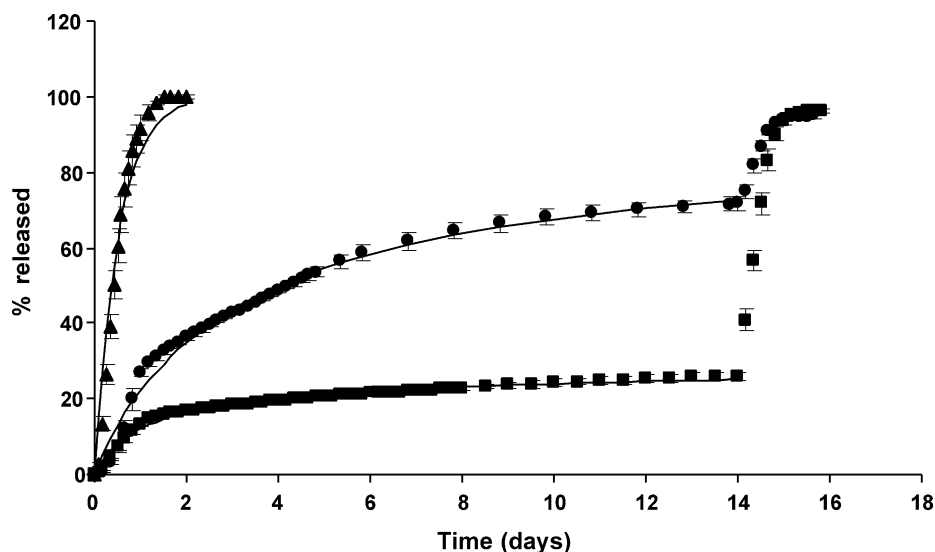


Fig. 2. In vitro cumulative release of carboxyfluorescein, free (triangles) or encapsulated in 100- (circles) or 280-nm (squares) egg phosphatidylcholine/cholesterol liposomes, from the chitosan/GP gel in PBS at 37 °C. After 14 days, Triton X-100 was added to the release medium to disrupt the liposomes still entrapped in the gel and release the remaining carboxyfluorescein. Each point represents the mean value \pm SEM ($n = 5$). The solid lines represent the simulated release kinetics. Reprinted with permission from Ref. [42].

injection of the paclitaxel-loaded hydrogel was as efficacious as four intravenous injections of the commercially available Taxol[®] formulation in inhibiting the growth of EMT-6 cancer cells, and proved to be less toxic (Fig. 3).

The chitosan/GP system was also evaluated as a tool for cartilage repair. Hoemann et al. [44] reported that primary calf chondrocytes could proliferate in solidified chitosan/GP solutions both in vitro and in vivo. Mechanical testing of 3-week aged in vitro implants demonstrated the initiation of functional matrix deposition. After injection into bone defects in rabbits, the chitosan/GP solution adhered to both bone and cartilage. Later, a hybrid implant, made of the chitosan/GP solution and whole blood, was developed to improve cartilage healing [45]. This new system, named

CarGel[®], was investigated in rabbits, sheep, and horses. The in vitro mixture of chitosan/GP with whole blood resulted in the accelerated formation of a more adhesive and voluminous clot scaffolding. In healing articular defects, specific repair responses were observed, including enhanced chemotaxis of bone marrow stromal cells, and temporarily increased vascularity of the repair tissue. No local or systemic toxic effects were seen in CarGel[®]-treated animals. Recently, human studies were undertaken with this new product.

Preliminary biocompatibility experiments showed that the local injection of chitosan/GP solutions in the rat hind paw led to an acute inflammatory response, which was inversely related to the chitosan's degree of deacetylation [46]. This response was substantially more important than

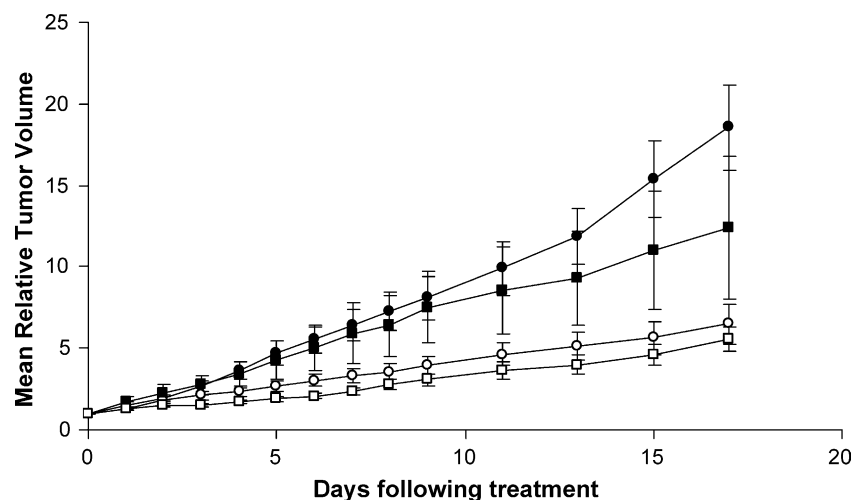


Fig. 3. In vivo antitumor effect in Balb/c mice bearing an EMT-6 tumor. Saline 0.2 ml/day intravenous \times 4 days (closed circles, $n = 8$), Taxol[®] 10 mg/kg/day intravenous \times 4 days (open circles, $n = 8$), chitosan/GP solution 10 μ l intratumoral (closed squares, $n = 8$) and paclitaxel/chitosan/GP solution (64 mg/ml paclitaxel) 10 μ l (40 mg/kg paclitaxel) intratumoral (open squares, $n = 8$). Reprinted with permission from Ref. [43].

that observed after the transdermal injection of similar solutions in the rat dorsal region [47], suggesting that the extent of the inflammatory response depended on the injection site. Hind paw injections may have elicited a more pronounced reaction because of differences in blood flow, tight geometry of the injection space, etc.

Jarry et al. [47,48] evaluated the effects of sterilization on chitosan/GP solutions. Initially, steam sterilization experiments were conducted on chitosan in the dry state. However, it was noted that, upon dissolution in water and the addition of GP, viscosity of the solutions increased dramatically compared to non-sterilized samples, compromising injectability of the system. Accordingly, it was decided to sterilize the polymer in solution. Steam sterilization of chitosan solutions induced molecular weight reduction, which led to a decrease in dynamic viscosity, gelling rate, and mechanical strength of the chitosan/GP hydrogels. Thus, this sterilization process may be considered when high mechanical performance is not critical or essential for implant function, such as in the case of controlled drug delivery.

In conclusion, chitosan/GP hydrogels constitute promising implants for pharmaceutical and bioengineering applications. Because of their high porosity, they are more adapted to the delivery of macromolecules and poorly water-soluble drugs.

3. *N*-isopropylacrylamide copolymers

Poly(*N*-isopropylacrylamide) (PNIPAM) (Fig. 1F) is a non-biodegradable polymer with a LCST $\sim 32^\circ\text{C}$ in water [17], and cross-linked gels of this material collapse around this temperature [49,50]. The PNIPAM LCST can be controlled by copolymerization with other monomers. The addition of hydrophilic monomers typically increases the LCST whereas the incorporation of more hydrophobic units has the opposite effect [51].

3.1. Poly(*N*-isopropylacrylamide-*co*-acrylic acid)

In the 1990s, Han and Bae [52] reported that an aqueous solution of high molecular weight P(NIPAM-*co*-AA) (Fig. 1G), synthesized in benzene (with 2 mol% AA in feed), showed reversible gelation at $\sim 32^\circ\text{C}$, above a critical concentration. Aqueous solutions of this copolymer did not just precipitate or result in a shrunken mass when the temperature was elevated above its LCST, like aqueous solutions of PNIPAM. A typical gelation process with a 5-wt% solution in phosphate-buffered saline (pH 7.4) is shown in Fig. 4A and B. The clear polymer solution (phase A) became cloudy as temperature increased to 27°C (cloud temperature). At this temperature, the solution was still freely mobile (phase B). With further heating up to the gelation temperature (35°C), the polymer solution became immobile (phase C). The initially formed gel was

translucent, and turned opaque with increasing temperature. At 43°C (gel-shrinking temperature), the gel started to shrink and expel water (syneresis). Upon cooling, it reverted to the sol state with no hysteresis between sol–gel and gel–sol temperatures (Fig. 4B). An interesting feature was that the gel did not dissolve or change its hydration state upon the addition of water (no swelling). The authors speculated that as temperature increases, polymer chains with lower AA content precipitate first, while those with higher AA content remain as partially collapsed globules or in a fully expanded coil state. The completely collapsed globules, entangled with the expanded coils, are thought to produce hydrophobic aggregates that form physical junctions.

P(NIPAM-*co*-AA) solutions were investigated as cell matrices in refillable bioartificial pancreas [53–58]. The idea originated from the observation that islets of Langerhans tend to malfunction at some point after transplantation and have to be replaced. Also, aggregation of the islets can lead to necrosis. The thermosensitive solutions were proposed as an extracellular matrix for islets placed in an immunoprotecting pouch installed in diabetic patients (Fig. 5). An islet/polymer solution can be injected into the pouch where the polymer forms a gel, immobilizing the islets. To replace the islets, the polymer matrix is cooled below the critical temperature, causing the gel to redissolve. The solution is then withdrawn, and a fresh islet/polymer suspension is injected. It was shown that the copolymer efficiently immobilized rat islets without impairing insulin secretion [53–55]. Compared to free islets, entrapment in the gel resulted in prolonged insulin secretion [55]. Moreover, P(NIPAM-*co*-AA)-entrapped rat islets responded faster and with greater magnitudes to changes in glucose concentration than islets in an alginate gel. The addition of glucagon-like peptide-1, a potent islet stimulant, was also investigated [56]. Its presence in the preparation significantly enhanced insulin secretion without affecting islet viability. Similarly, a hemoglobin–PEO conjugate was used, as it is believed that the low functionality and viability of immunoprotected islets are closely associated with low oxygen tension [57]. Insulin secretion functions, as well as islet viability, were significantly enhanced by the addition of hemoglobin. The same approach was later taken to develop a biohybrid artificial liver [59]. Hepatocytes cultured as spheroids in the thermoreversible matrix exhibited greater viability and enhanced liver-specific functions vs. control cells. These studies demonstrated the potential of the system as a temporary cell-supporting matrix, but the non-biodegradability of the polymer may limit its biomedical applications.

3.2. Poly(*N*-isopropylacrylamide)/poly(ethylene oxide)

A new family of polymers that self-assemble to form gels in a thermoreversible fashion has been proposed recently by Lin and Cheng [60]. It consists of block linear and star

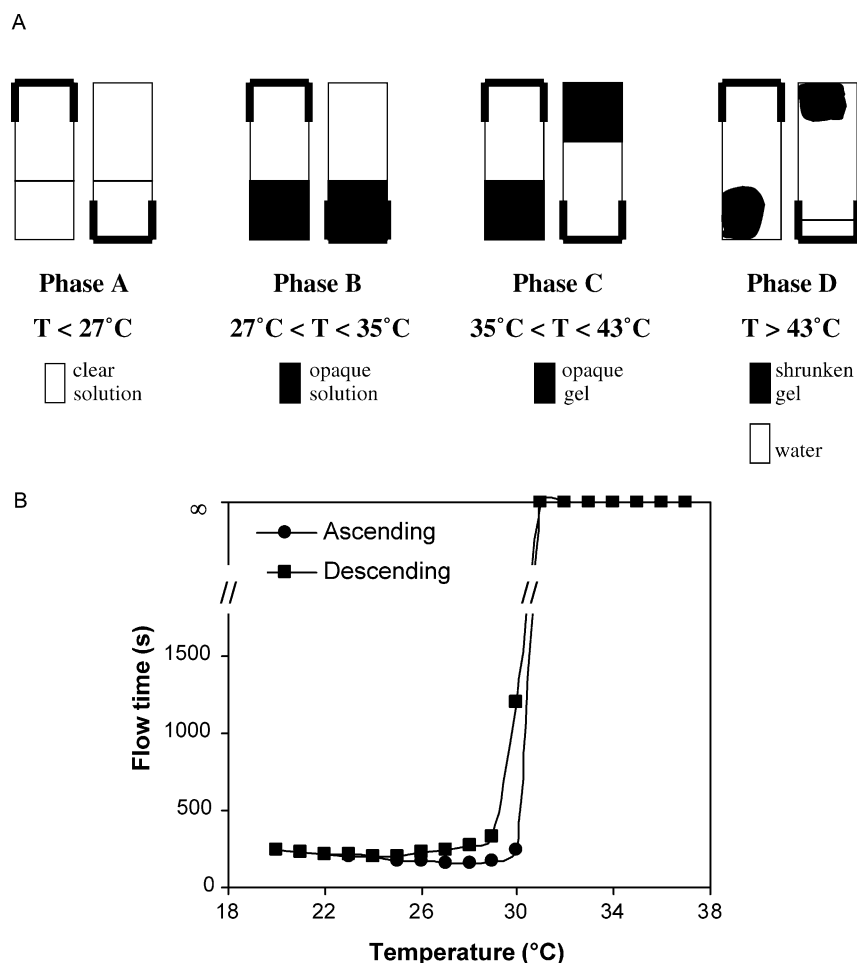


Fig. 4. Phase transitions (A) and viscosity (flow time through a Cannon–Fenske viscometer) of an aqueous solution of P(NIPAM-co-AA) as a function of temperature. Adapted with permission from Ref. [52].

copolymers with a central hydrophilic PEO segment and temperature-responsive PNIPAM terminal segments. Linear and star copolymers of PEO and PNIPAM form liquid aqueous solutions at room temperature that transform to relatively strong elastic gels upon heating. Multiple-arm copolymers yield gels via physical cross-links between aggregates of PNIPAM segments, whereas diblock copolymers gel by a micellar aggregation mechanism (see Section 4). The rheological properties of the hydrogels were found to depend on molecular architecture, with the star structure having four PNIPAM terminal segments showing superior qualities. These copolymer solutions presented low to moderate injection viscosities, high gel strengths, low degrees of syneresis, and rapid gelation kinetics. So far, the systems have not been investigated for biomedical purposes.

4. Poloxamer systems

4.1. Poloxamer (Pluronic®)

The poloxamers (Fig. 1H) consist of more than 30 different non-ionic surface-active agents. These polymers

are ABA-type triblock copolymers composed of PEO (A) and PPO units (B). The poloxamer series covers a range of liquids, pastes, and solids, with molecular weights and ethylene oxide-propylene oxide weight ratios varying from 1100 to 14,000 and 1:9 to 8:2, respectively. Concentrated aqueous solutions of poloxamer form thermoreversible gels.

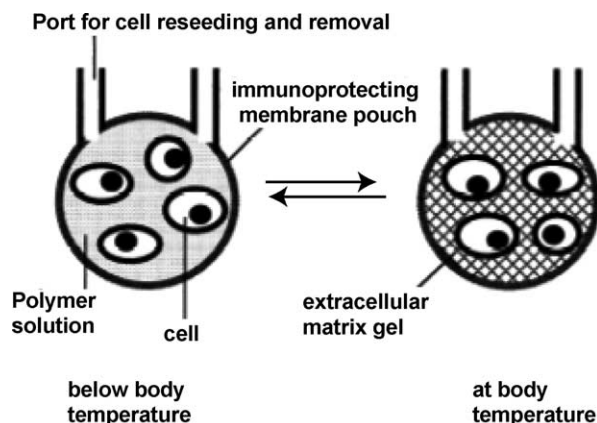


Fig. 5. Schematic diagram illustrating the concept for a refillable cell delivery system with a thermally reversible extracellular matrix. Reprinted with permission from Ref. [54].

The gelation mechanism of poloxamer solutions has been investigated extensively, but is still being debated. Ultrasonic velocity, light-scattering and small-angle neutron scattering measurements of aqueous poloxamer solutions have clearly indicated a micellar mode of association [61–66]. Micelle formation occurs at the critical micellization temperature as a result of PPO block dehydration [63,67]. With increasing temperature, micellization becomes more important, and at a definite point, micelles come into contact and no longer move. In addition, the formation of highly ordered structures, such as cubic crystalline phase, has been proposed as the driving force for gel formation [20,64–66], but this hypothesis has been questioned recently. Thus, packing of micelles and micelle entanglements may be possible mechanisms of poloxamer solution gelation with increased of temperature [68].

Poloxamer 407 (Pluronic® F127) was found to gel at a concentration of 20 wt% at 25 °C, which is less than that of the other members of the poloxamer series. At room temperature (<25 °C), the solution behaves as a mobile viscous liquid, which is transformed into a semi-solid transparent gel at body temperature (37 °C). Preliminary toxicity data indicate that this copolymer is well tolerated [69]. Taken together, these results have prompted the use of poloxamer 407 in the design of medical, pharmaceutical, and cosmetic systems. Early studies evaluated poloxamer 407 thermosensitive solutions for the treatment of burns [69], topical administration of anticancer agents [70], and sustained delivery of drugs after extravascular parenteral injection [71]. Over the past 15 years, this copolymer has been investigated extensively for various applications, and it is not the intent of this paper to review them all. Some recent applications are reported in Table 1 and discussed below. After parenteral injection, poloxamer gels can prolong drug release compared to solutions, but the delivery period rarely exceeds a few days [72–76]. This characteristic makes poloxamer gels interesting for short-term therapies like pain management [74], infection treatment [73,76], and fertility control [75]. Besides injectables, other administration routes have been evaluated, such as rectal [77,78], vaginal [79,80], transdermal [81,82] and ophthalmic [83,84]. Poloxamer formulations generally increased drug residence time at application sites, resulting in improved bioavailability and efficacy (Fig. 6).

Poloxamer 407 is usually regarded as non-toxic. After intramuscular injection in rabbits, poloxamers 238 and 407 displayed musculoiritancy/toxicity comparable to that of traditional intramuscular vehicles, such as saline and peanut oil [85]. However, other studies have reported systemic side effects. Wout et al. [86] demonstrated that poloxamer 407 injected intraperitoneally into rats (1.5 g/kg) resulted in sustained hypercholesterolemia and hypertriglyceridemia (>96 h). Palmer et al. [87] obtained similar results in mice. It was suggested that the predominant mechanism for this effect was inhibition of heparin-releasable lipoprotein lipase [88]. Blonder et al. [89] examined whether lower but

clinically useful doses of poloxamer 407 gels induced hyperlipidemia in rabbits. The highest dose (137.5 mg/kg) significantly increased serum triglycerides and cholesterol in both male and female rabbits, with peak values observed 2 days after injection. The lower doses (5.5–27.5 mg/kg) did not alter serum lipids. Hence, the amount of administered polymer should be kept to a minimum, especially when repeated dosing is required.

Potential drawbacks of poloxamer gels include their weak mechanical strength, rapid erosion (i.e. dissolution from the surface), and the non-biodegradability of PEO-PPO-PEO, which prevents the use of high molecular weight polymers that cannot be eliminated by renal excretion. To circumvent the biodegradability issue, new polymers were synthesized by linking together a few (usually 3) poloxamer 407 ‘monomers’ via degradable carbonate linkage [90]. As the carbonate linkages were hydrolyzed under physiological conditions, the hydrogel degraded into soluble poloxamer 407 units and carbonate. Variation of the polymer concentration enabled modification of the gel’s dissolution time (25–80 days). Another interesting feature is that oligomerization decreased the critical gel concentration by two-fold. To address their poor mechanical properties, Cohn et al. [91] proposed two different strategies: (i) to polymerize poloxamers using hexamethylene diisocyanate as a linker; and (ii) to use phosgene to covalently bind PEO and PPO chains. These new materials achieved viscosities that were at least 15 times higher than poloxamer 407 at 37 °C. Moreover, the critical gel concentration was lowered to 5 wt%. Also, a 30% poly(poloxamer 407)₄ gel delivered drug over a 40-day-period vs. 7 days for poloxamer 407.

4.2. Poloxamer/poly(acrylic acid)

High molecular weight poly(acrylic acid) (PAA) is a bioadhesive polymer known to adhere to the hydrated mucosal cells coating the eye, nose, lungs, gastrointestinal tract, and vagina. It is often incorporated into drug delivery systems that come in contact with mucosal surfaces in order to prolong the residence time. As described earlier, random copolymers of NIPAM and AA can gel with an increase in temperature (Section 3.1). However, thermosensitivity is lost at physiological pH when the AA content is increased so as to obtain bioadhesive properties. To circumvent this problem, graft copolymers were developed [92]. At pH 7.4, the graft copolymers exhibited phase transition at a temperature similar to that of PNIPAM. Nevertheless, NIPAM copolymers are not generally recognized as safe (GRAS). This fact prompted investigators to synthesize similar copolymer structures using GRAS polymers. Since poloxamer and PAA components have approved regulatory status, it is expected that their graft-copolymers would be safe. Synthesis of the graft copolymer, PAA-g-poloxamer (Fig. 1J), was reported by Hoffman and co-workers [93–95]. The proposed synthetic route involved a three-step procedure with several intermediate steps of purification,

Table 1

Poloxamer temperature-sensitive solutions for drug delivery applications: some examples since 1997

Poloxamer	Concentration (wt%)	Drug	Objective of the study	Ref.
407	36	Recombinant human growth hormone	Controlled release of human growth hormone following intramuscular or subcutaneous administration.	[72]
407	20 or 30	Insulin	Subcutaneous delivery of peptides and proteins having short half-lives.	[132]
407	25	Vancomycin	Prolonged residence time of vancomycin in a body site with a high infection risk.	[73]
407	25	Ibuprofen	Controlled release of ibuprofen for epidural analgesia.	[74]
407	20	Paclitaxel	Intratumoral administration of paclitaxel.	[133]
407	25	Deslorelin or GnRH	Intramuscular sustained release of deslorelin and GnRH to induce the release of luteinizing hormone and formation of luteal tissue in cattle.	[75]
407	25, 30 and 35	Ceftiofur	Sustained release gel formulation of ceftiofur for treating foot infections in cattle.	[76]
407 and/or, 188 and additives	15, 15 and 20	Acetaminophen	Increased bioavailability using an in situ gelling and mucoadhesive liquid suppository.	[77]
407 and 188 and additives	15	Propranolol	Increased bioavailability using an in situ gelling and mucoadhesive liquid suppository.	[78]
407, 188	15, 15 and 20	Clotrimazole	Prolonged antifungal effects using an in situ gelling and mucoadhesive vaginal gel.	[79,80]
407 and thickening agents	15, 20 or 25	Timolol maleate	Enhanced ocular bioavailability of timolol maleate.	[83]
407, 188	21	None	Development of a thermosetting gel with a suitable phase transition temperature for ocular delivery.	[84]
407		Piroxicam	Enhanced efficacy of piroxicam following percutaneous absorption.	[81]
	46	Fentanyl	Poloxamer gels as release vehicles for percutaneous administration of fentanyl.	[82]

making the process potentially problematic on an industrial scale. Subsequently, the synthesis of another graft copolymer, i.e. poloxamer-*g*-PAA (Fig. 11), was described by Bromberg et al. [96–98]. This single-step synthetic procedure was reproducible and scaleable. Work on this copolymer (Smart Hydrogel™) has been reviewed recently by Bromberg and Ron [99]. While the PNIPAM-*g*-PAA graft copolymers precipitated out of solutions when temperature was raised above the LCST, dilute solutions (0.5–3 wt%) of PAA-*g*-poloxamer and poloxamer-*g*-PAA formed clear gels when the temperature increased from 4 to 37 °C at pH 7.4 [94,97,98] (Fig. 7). Moreover, the critical gel concentration was considerably lower than that of the parent poloxamer. Bromberg thoroughly studied the rheological properties of poloxamer-*g*-PAA hydrogels and showed that temperature-induced sol–gel transition and gel strength were concentration-dependent [100–102].

An increase in polymer concentration resulted in lower transition temperature and higher gel strength. Besides polymer concentration, pH and salts influenced gelation properties [102]. With rising pH, the onset of gelation was shifted to lower temperatures, and gel strength increased.

The data obtained by Bromberg suggested that gelation was driven by the formation of micelles that act as thermoreversible cross-links. The onset of gelation in poloxamer-*g*-PAA solutions occurred approximately at the critical micellization temperature of the parent poloxamer [97]. Polymer aggregation indeed coincided with the onset of the relative viscosity increase [101]. A negative change in heat capacity upon gelation suggested a decrease in the exposure of PPO segments to water [102].

The bioadhesive nature of this system [103] makes it interesting for a wide variety of applications. Hydrogels of

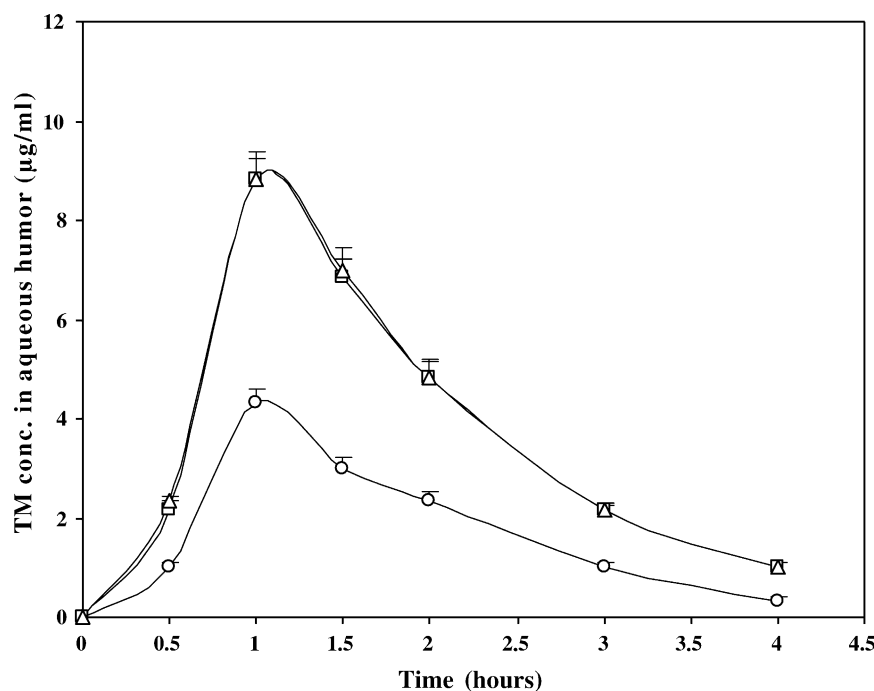


Fig. 6. Timolol concentration in aqueous humor after instillation of timolol aqueous solution (circles), timolol-poloxamer solution (squares) and timolol-poloxamer-methylcellulose solution (triangles). Reprinted with permission from Ref. [83].

poloxamer-g-PAA have been proposed for vaginal [104] and topical [105] drug administration. The pharmacokinetic profile of estradiol after vaginal delivery of poloxamer-g-PAA formulation was equivalent to that of Estrace[®] vaginal

cream, which contains five times more drug. Potts et al. [106] examined this hydrogel in the treatment of gastro-oesophageal reflux by measuring its adherence to the oesophageal mucosa. This study revealed that 15% of

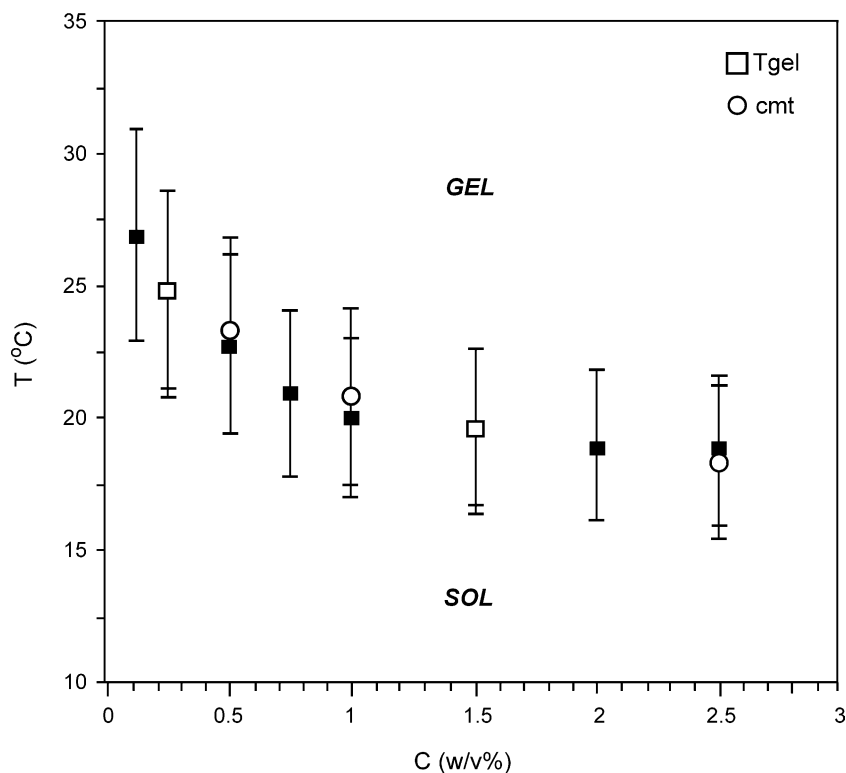


Fig. 7. Phase diagram for aqueous solutions of poloxamer-g-PAA at pH 7.0. T_{gel} and cmt stand for the gel point and critical micellization temperature determined by the Winter–Chambon method and DSC, respectively. Reprinted with permission from Ref. [102].

the administered dose displayed prolonged retention in the oesophagus. In another investigation, it was shown that Smart Hydrogel™ could sustain the release of luteinizing hormone-releasing hormone (LHRH) and human insulin [107]. At a polymer concentration of 5%, the release kinetics of LHRH followed a zero-order rate. Finally, preliminary animal toxicological studies showed the non-toxic nature of the copolymer administered topically, orally or to the eye [99].

5. Poly(ethylene oxide)/poly(D,L-lactic acid-co-glycolic acid)

Jeong and co-workers described different thermosensitive, biodegradable hydrogels based on poly(lactic acid). Block copolymer solutions of PEO and poly(L-lactic acid) were shown to be in the sol state at 45 °C, and in the gel state at body temperature [108]. However, the need to heat the solution limits the nature of the drugs that can be incorporated in this delivery system, and makes the injection procedure not practical. Later, PEO-*b*-(D,L-lactic acid-co-glycolic acid)-*b*-PEO (PEO-PLGA-PEO) (Fig. 1K) triblock copolymer solutions were found to form, at room temperature, a free-flowing sol that became a transparent gel at 37 °C [109,110]. PEO-PLGA-PEO (550-2810-550) exhibited a critical gel concentration of approximately 16 wt%.

PEO-PLGA-PEO polymers are amphiphilic and yield micelles in aqueous environments with the hydrophilic PEO occupying the outer shell region, and the hydrophobic PLGA constituting the inner core. Micelle formation was indeed confirmed by ¹³C-NMR and dye solubilization studies [109]. It was shown that the gels formed via a micellar aggregation mechanism. Abrupt changes in micellar diameter and aggregation number were demonstrated above 30 °C, and the second virial coefficient decreased sharply at this temperature, indicating a sudden increase in polymer–polymer attraction [109]. Other studies established that micelle–micelle clusters were formed through hydrophobic interactions between core phases [111]. The latter mechanism differentiates these copolymers from poloxamers where gel formation is only associated with simple micellar entanglements. The gelling properties are influenced by PLGA or PEO proportions. The more hydrophobic the polymer, the lower the temperature and polymer concentration required to achieve the desired liquid crystalline structure [110].

In vitro studies revealed that the gel could sustain the release of ketoprofen (hydrophilic drug) and spironolactone (hydrophobic drug) for 2 weeks and 2 months, respectively [112]. The subcutaneously injected solution formed a hydrogel in situ [113]. The latter maintained a three-dimensional shape at the implant site, indicating that gelation was fast. The implant was strong enough to be easily taken out with forceps and persisted for more than

1 month with little or no tissue irritation at the injection site. As opposed to poloxamers, PEO-PLGA-PEO gels do not erode rapidly. Furthermore, due to their PLGA segments, they can be hydrolyzed in vivo.

Since the transition temperature of the triblock copolymers is quite sensitive to PEO length, it is difficult to modify polymer structure and still maintain gelation temperature around 37 °C. To overcome the molecular weight limitation, Jeong et al. [114–117] developed graft copolymers based on PEO and PLGA. As with the PEO-PLGA-PEO system, PEO-*g*-PLGA (Fig. 1L) and PLGA-*g*-PEO (Fig. 1M) micellized in water [114–116]. The critical gel concentrations of both polymers were approximately 16 [114] and 22 wt% [116], respectively. The applicability of this new material as an in situ-forming device was assessed after subcutaneous injection into rats [116]. Again, the implant was round-shaped, indicating fast gelation kinetics. In vivo, the PLGA-*g*-PEO depot persisted for about 3 months, whereas PEO-*g*-PLGA disappeared within a week. By mixing two polymers of PEO-*g*-PLGA and PLGA-*g*-PEO, the duration of depot could be adjusted between 1 week and 3 months. The grafted copolymer systems were evaluated for sustained insulin release and as a cell delivery matrix in cartilage repair [117]. Insulin depots prepared from a 50/50 mixture of PEO-*g*-PLGA and PLGA-*g*-PEO showed a hypoglycemic effect over 5 days vs. 16 days for PLGA-*g*-PEO. Injection of a chondrocyte cell suspension in PLGA-*g*-PEO into a cartilage defect effectively promoted cartilage repair in rabbits.

Low molecular weight PLGA-PEO-PLGA copolymers also exhibit reverse thermal gelation properties at concentrations between 10 and 30 wt% [118]. This system, known as ReGel®, was evaluated as a pharmaceutical vehicle by Zentner et al. [119]. In vivo biodegradation studies in rats revealed that the formulation was completely resorbed from the injection site within 4–6 weeks. In vitro release experiments showed sustained paclitaxel release over 50 days. This was in sharp contrast to the complete release of paclitaxel from poloxamer 407 hydrogel within 1 day. Direct intratumoral injection of paclitaxel-loaded ReGel® resulted in slow drug clearance from the injection site with minimal distribution into any organ. The efficacy of ReGel®/paclitaxel (OncoGel®) against human breast tumor xenografts (MDA231) was also superior to that achieved with a maximum tolerated dose of the commercial paclitaxel product (i.e. Taxol®). Moreover, the ReGel®/paclitaxel formulation was less toxic as treated animals showed no drug-related adverse effects, whereas in the Taxol® group, there was weight loss and two acute toxic deaths within 2 days of dosing. OncoGel® is MacroMed's lead product; it entered Phase II studies in the second quarter of 2003 [120]. The ReGel® system has also been tested for the parenteral delivery of insulin [121]. After one injection in rats, elevated plasma insulin levels were maintained for at least 15 days (Fig. 8).

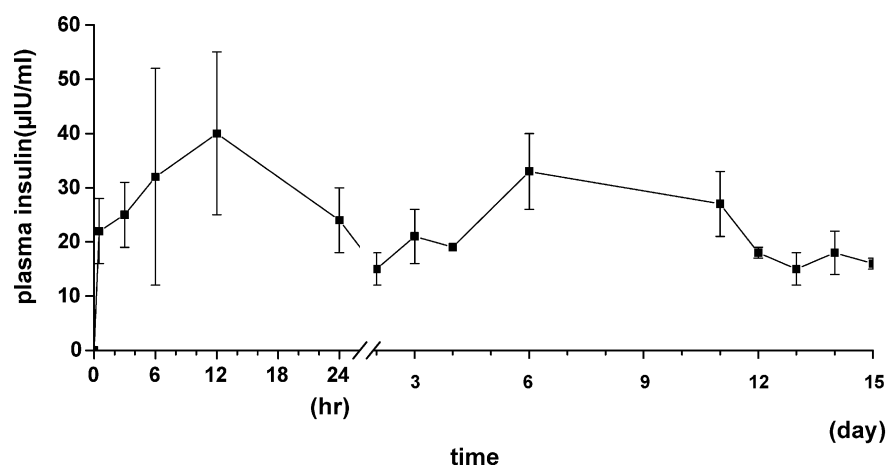


Fig. 8. Plasma insulin levels of Sprague–Dawley rat after subcutaneous injection of the insulin/ReGel[®] formulation. Reprinted with permission from Ref. [121].

6. Thermosensitive liposomes as a physical barrier between reactive species

In a series of studies, Messersmith and co-workers [122–126] exploited a rise in temperature to initiate a reaction cascade leading to the formation of a biomaterial

in situ. Their approach was based on the use of temperature-sensitive liposomes to compartmentalize reactive species (Fig. 9A). The physical barrier imposed by the vesicle membrane prevented the chemical reaction from proceeding. Destabilization of the lipid membrane upon an increase in temperature triggered

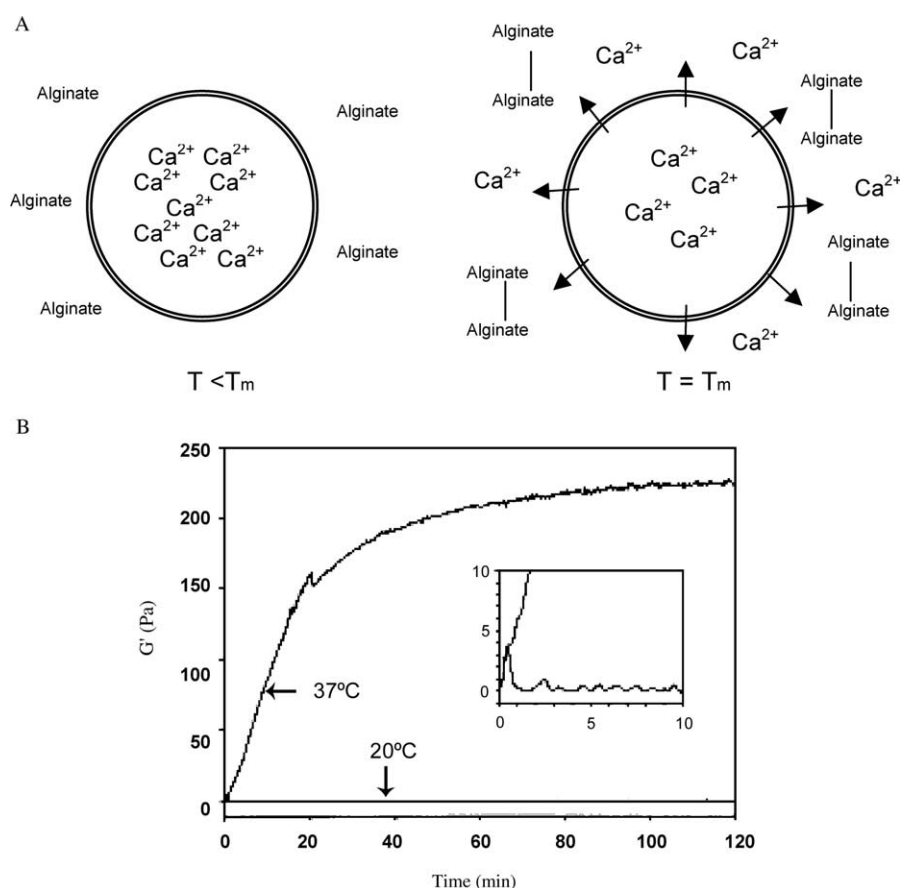


Fig. 9. (A) Schematic illustration of thermally triggered alginate cross-linking. Adapted with permission from Ref. [122]. (B) Thermally triggered gelation of TGase/fibrinogen/thermally sensitive, calcium-loaded liposome precursor fluid. Reprinted with permission from Ref. [123].

the reaction as reactive species escaped from the liposomes. The same strategy can be adopted to design thermoresponsive gels.

Alginates are linear, water-soluble polysaccharides composed of 1,4-linked β -D-mannuronic and α -L-glucuronic acid units. One of the main biofunctional characteristics of alginates is their ability to form transparent or translucent gels in the presence of multivalent ions. Thus, thermoresponsive calcium-loaded liposomes were prepared and mixed with an alginate solution [123]. Ca^{2+} sequestration within the liposomes prevented the ionic cross-linking of the polymer. The Ca-liposome/alginate mixture remained fluid when stored at 20 °C. Gelation occurred upon heating the system above the liposome phase transition temperature due to the leakage of entrapped Ca^{2+} . At room temperature, however, after only 1 day of storage, a gradual increase in viscosity was observed, which eventually led to full gelation within 4–5 days. It was found that alginate triggered slow release of calcium from the liposomes at 20 °C. Although the lack of stability imposes constraints on the storage time of the mixture, it does not necessarily preclude the use of such a system, since the two components can be mixed prior to injection.

Many biological processes are regulated by Ca^{2+} concentration, indicating that Ca^{2+} release can be exploited to produce different types of biomaterials. Protein-based hydrogels were prepared by taking advantage of Ca^{2+} -dependent enzymes to catalyze enzyme-mediated crosslinking of macromolecular substrates. One such family of Ca^{2+} -dependent proteins are the transglutaminases. These enzymes catalyze the formation of crosslinks between glutamine- and lysine-containing proteins. Calcium-loaded, thermally sensitive liposomes were used to activate hrFXIII, a calcium-dependent enzyme that catalyzes the crosslinking of fibrin during blood coagulation [123,124]. When calcium-loaded liposomes, fibrinogen, and hrFXIII were combined and heated to 37 °C, gelation occurred within 10 min (Fig. 9B). Sandborn et al. [125] reported a system comprising thermosensitive, calcium-loaded liposomes, hrFXIII, thrombin and an enzymatic substrate based on a four-arm PEO terminated with a 20mer peptide sequence derived from the γ -chain of fibrin. Upon heating the precursor solution to 37 °C, a solid, enzymatically crosslinked hydrogel formed within 9 min. Gelation was not reversible as the material remained in a gelled state after cooling to room temperature. Another study investigated the potential of the system to trigger the self-assembly of FEK16 [126], a 16-amino acid peptide that is highly soluble in pure water, but forms self-assembled aggregates in the presence of NaCl, KCl, and CaCl_2 . Hydrogel formation was achieved when temperature-sensitive, calcium-loaded liposomes were suspended in aqueous solutions of FEK16 and subsequently warmed to 37 °C. The main advantage of using peptide substrates as hydrogel crosslinks lies in their biodegradability.

However, a possible immune response to the implant is a potential concern that should be addressed in the future.

7. Miscellaneous

7.1. Poly(organophosphazene) derivatives

Recently, poly(organophosphazene) (PPZ) derivatives were shown to exhibit sol–gel phase transitions as a function of temperature. PPZ bearing methoxy-PEO and amino acid esters as substituents were synthesized by Song et al. (Fig. 1N) [127,128]. The polymers were hydrolytically degradable and displayed a LSCT in the 25.2–98.5 °C range. The same group demonstrated that oligomeric cyclophosphazenes with proper orientation of substituents were also thermosensitive (Fig. 1P) [129]. PPZ bearing α -amino- ω -methoxy-PEO and hydrophobic L-isoleucine ethyl ester as side groups exhibited reversible sol–gel transition between 29 and 61 °C depending on the structure (Fig. 1O) [130]. Gelation was attributed to hydrophobic interactions between the side-chain fragments ($-\text{CH}(\text{CH}_3)-\text{CH}_2\text{CH}_3$) of L-isoleucine ethyl ester.

7.2. Poly(1,2-propylene phosphate)

Lately, a new injectable temperature-sensitive hydrogel system was proposed by Wang et al. [131]. The system is based on a synthetic polyanion, poly(1,2-propylene phosphate) (Fig. 1Q), that can be crosslinked by calcium ions. Solutions of poly(1,2-propylene phosphate) in water did not exhibit temperature-dependent phase transition. In the presence of CaCl_2 , the polymer solutions remained liquid at room temperature, but gelled rapidly when heated. The sol–gel transition temperature was a function of polymer and Ca^{2+} concentrations. The in vitro release study of lysozyme followed zero-order kinetics after a lag time of 1 h. Release was accompanied with decross-linking of the gel, which was most likely due to Ca^{2+} diffusion into the buffer.

8. Conclusion

Over the last decade, an impressive number of novel, thermosensitive, in situ gel-forming solutions have been described in the literature. Each system has its own advantages and drawbacks. The choice of a particular hydrogel depends on its intrinsic properties and envisaged therapeutic use. For instance, the formation of a transparent gel is particularly important when ophthalmic applications are considered. Non-biodegradable gels could prove useful for administration routes other than parenteral. Poloxamer hydrogels perhaps represent the most extensively studied systems. However, despite the clinical acceptance of poloxamers as solubilizer and thickening agents, these polymers have not met initial expectations as pharmaceutical

and biomedical implants, mainly due to their non-biodegradability and inability to provide sustained drug delivery over more than just a few days. Recent developments in the design of poloxamer derivatives showed that some of these problems could be worked out. Polysaccharides usually demonstrate good biocompatibility and/or biodegradability, and their solutions are thermosensitive at low polymeric concentrations. However, these systems may not be adapted for the sustained release of hydrophilic, low molecular weight drugs because their large, water-filled pores permit rapid diffusion. On the other hand, they provide adequate scaffolds for cell growth and tissue repair. PEO/PLGA hydrogels are particularly attractive systems for pharmaceutical applications. They are biodegradable and generally have a good safety profile. Their composition can be tailored to provide drug delivery over weeks or months after parenteral extravascular administration.

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