Published in final edited form as:
Eur J Pharm Biopharm. 2008 January ; 68(1): 34–45. doi:10.1016/j.ejpb.2007.02.025.

Thermoresponsive hydrogels in biomedical applications - a review

Leda Klouda and **Antonios G. Mikos***
Department of Bioengineering, Rice University, PO Box 1892, MS-142, Houston, TX 77251-1892, USA

Abstract

Environmentally responsive hydrogels have the ability to turn from solution to gel when a specific stimulus is applied. Thermoresponsive hydrogels utilize temperature change as the trigger that determines their gelling behavior without any additional external factor. These hydrogels have been interesting for biomedical uses as they can swell *in situ* under physiological conditions and provide the advantage of convenient administration. The scope of this paper is to review the aqueous polymer solutions that exhibit transition to gel upon temperature change. Typically, aqueous solutions of hydrogels used in biomedical applications are liquid at ambient temperature and gel at physiological temperature. The review focuses mainly on hydrogels based on natural polymers, *N*-isopropylacrylamide polymers, poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) polymers as well as poly(ethylene glycol)-biodegradable polyester copolymers.

Keywords

Thermosensitive hydrogels; in situ gelation; drug delivery; tissue engineering; lower critical solution temperature

1. Introduction

Hydrogels are composed of hydrophilic homopolymer or copolymer networks and can swell in the presence of water or physiological fluids. Chemical crosslinks (covalent bonds) or physical junctions (e.g. secondary forces, crystallite formation, chain entanglements) provide the hydrogels' unique swelling behavior and three-dimensional structure [1, 2, 3]. Hydrogels have been a topic of extensive research in the past decades and their properties as for example their high water content and the possible control over the swelling kinetics make them very attractive for biomedical applications. More specifically, *in situ* forming hydrogels can provide a means for simple, "custom-made" therapeutics and diagnostics. A polymer solution can be prepared and allowed to gel *in situ*, after photopolymerization [4, 5], chemical crosslinking [6, 7], ionic crosslinking [8] or in response to an environmental stimulus such as temperature, pH or ionic strength of the surrounding medium [9, 10]. Hydrogels that respond to temperature change are the subject of this review. Their

*Corresponding author. Department of Bioengineering, Rice University, PO Box 1892, MS-142, Houston, TX 77251-1892, USA, Tel.: +1 713 348 5355; fax: +1 713 348 4244. mikos@rice.edu (A.G. Mikos).

sensitivity to the thermal environment is useful as temperature is the sole stimulus for their gelation with no other requirement for chemical or environmental treatment, and can be thus produced e.g. upon injection to the body, when temperature is increased from ambient to physiological.

The phenomenon of transition from a solution to a gel is commonly referred to as sol-gel transition. Some hydrogels exhibit a separation from solution and solidification above a certain temperature. This threshold is defined as the lower critical solution temperature (LCST). Below the LCST, the polymers are soluble. Above the LCST, they become increasingly hydrophobic and insoluble, leading to gel formation. In contrast, hydrogels that are formed upon cooling of a polymer solution have an upper critical solution temperature (UCST) [10]. The sol-gel transition of thermosensitive hydrogels can be experimentally verified by a number of techniques such as spectroscopy [11–13], differential scanning calorimetry (DSC) [11, 12] and rheology [11].

There are various mechanisms behind thermogelation in aqueous solutions, and for some polymers they are still a topic of debate. Many polymers show a decrease in solubility that is attributed by changes in the overall hydrophilicity of the polymer chains upon temperature change. When a polymer is dissolved in water, there are three types of interactions that take place: between polymer molecules, polymer and water and between water molecules. For polymers exhibiting an LCST, temperature increase results in a negative free energy of the system which makes water-polymer association unfavorable, facilitating the other two types of interactions. This negative free energy (ΔG) is attributed to the higher entropy term (ΔS) with respect to the increase in the enthalpy term (ΔH) in the thermodynamic relation $\Delta G = \Delta H - T\Delta S$. The entropy increases due to water-water associations which are the governing interactions in the system. This phenomenon is the so-called hydrophobic effect [11, 14, 15]. Polymer micelle packing [16] and coil to helix transition causing network formation [17] are examples of the conformational changes that take place at the critical solution temperature. All of these result in a reversible physical linking of the polymer chains, and gels can therefore return to solution after the thermal stimulus that caused their gelation is removed.

This article reviews the applications of thermosensitive hydrogels in fields of interest for pharmaceutical and biomedical scientists and engineers. It emphasizes mainly the use of hydrogels based on natural polymers, *N*-isopropylacrylamide (NiPAAM) polymers, poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO) as well as poly(ethylene glycol) (PEG)-biodegradable polyester copolymers. As there are some excellent reviews [18, 19] summarizing the literature on thermosensitive hydrogels published in the past years, the scope of this work is to cover the advances in the field from 2004 until today (2006).

2. Natural polymers and derivatives

Many natural polymers have been shown to exhibit gelation upon temperature change. Researchers have used them alone or in combination with synthetic polymers to fabricate thermally responsive hydrogels with desired properties.

2.1 Polysaccharides

2.1.1 Cellulose derivatives—Cellulose is a natural polysaccharide which is insoluble in water. Substitution of the hydroxyl groups on cellulose with more hydrophobic units as methyl or hydroxypropyl groups renders the originally insoluble cellulose water soluble [11]. Methylcellulose (MC) is a cellulose derivative that has been extensively investigated for biomedical applications. It has thermoreversible gelation properties in aqueous solutions, gelling at temperatures in the range of 60–80°C and turning into a solution upon cooling [11,

20]. Liu et al. [21] have grafted methylcellulose with the synthetic *N*-isopropylacrylamide (NiPAAm), combining the thermogelling properties of both materials. It was possible to prepare fast reversibly thermogelling hydrogels by adjusting the ratios of the two components. They reported that a low percentage of methylcellulose decreases the LCST as compared to pNiPAAm, but with a high MC ratio the LCST increases. They also found that addition of MC to NiPAAM polymers enhances the mechanical strength of the hydrogel with no syneresis.

Another interesting approach towards a thermosensitive hydrogel system based on methylcellulose was recently reported by Stabenfeldt et al. [22]. As it had been shown before [23] that methylcellulose showed low protein adsorption and cell adhesion, Stabenfeldt et al. functionalized methylcellulose with the protein laminin, aiming towards the creation of a bioactive scaffold for neural tissue engineering. In order to facilitate laminin tethering, methylcellulose was oxidized previous to functionalization. They reported that the laminin-functionalized oxidized methylcellulose (OXMC+LN) hydrogel promoted neuronal cell adhesion and showed higher cell viability rates than methylcellulose (MC), oxidized methycellulose (OXMC) or laminin-functionalized methylcellulose (MC+LN) (figure 2). Moreover, the concentration could be adjusted so that the hydrogel exhibits a lower critical solution temperature slightly below physiological temperature.

2.1.2 Chitosan—Chitosan is produced with the deacetylation of chitin, which can be found in the outer skeleton of shrimp and insects, among others. We would like to refer the reader to the excellent review by Gariépy and Leroux [19] which covers the developments of thermosensitive chitosan-polyol salt hydrogels until 2004. More recently, Bhattarai et al. [24] incorporated poly(ethylene glycol) (PEG) into chitosan and were able to form a thermoreversible hydrogel with no additional crosslinking agents. Moreover, PEG grafting improved the solubility of chitosan in water, and the gelation was found to be possible in physiological pH values. The same group [25] evaluated the PEG-grafted chitosan for controlled drug release *in vitro*. Using albumin as a model protein, an initial burst release was observed, which was followed by a steady release from the hydrogel for about three days. After this time, the remaining albumin could not be released from the hydrogel until the gel matrix was dissolved in the media. When the PEG-grafted chitosan was crosslinked *in situ* with genipin, a crosslinking agent with low cytotoxicity, quasi-linear drug release was possible for up to 40 days; however the hydrogel lost its thermoreversibility at 37°C.

Chitosan-based hydrogels have been also investigated as potential cell carriers for tissue engineering applications. A copolymer of NiPAAm and water-soluble chitosan was tested for chondrogenic differentiation of human mesenchymal stem cells (hMSC). The hydrogel showed a stable gelation at 37°C and differentiation of hMSC into chondrocytes was observed, both *in vitro* and *in vivo*. This hydrogel could be used for a minimally invasive treatment of vesicouretral reflux with an endoscopic procedure through a single injection [26]. Dang et al. [27] modified chitosan by adding hydroxybutyl groups to its hydroxyl and amino reactive sites, rendering it water soluble and thermally responsive. The resulting hydrogel could gel within seconds after exposure to physiological temperature and was sufficiently strong to be handled with, as for example to measure mechanical properties. Upon cooling, the gel returned to liquid state. Having as an ultimate goal the treatment of degenerative disc disease, the hydrogel's interaction with hMSC and cells derived from the intervertebral disc was tested. The cells were able to proliferate and produce extracellular matrix when encapsulated in the hydrogels for a period up to two weeks.

A chitosan-glycerophosphate salt (GP) hydrogel was recently tested for its potential in neural tissue engineering. This thermally responsive hydrogel, as developed initially by [28], has been shown to have good biocompatibility *in vitro*, but it hadn't been yet tested with

nerve cells. Crompton et al. [29] compared the hydrogel to polylysine-functionalized chitosan-GP, with the hypothesis that the peptide polylysine might improve neuronal adhesion and neurite outgrowth. When neurons were grown in a two-dimensional culture, the functionalized hydrogel did not seem to significantly affect cell survival as compared to chitosan-GP, but it was observed that increasing concentrations of polylysine inhibited neurite outgrowth. However, when the cells were cultured in a three-dimensional functionalized gel, a geometry that is more representative of the extracellular matrix (ECM) environment, in certain polylysine concentrations more cells were viable than on non-modified chitosan-GP. The authors concluded that polylysine-chitosan-GP may be a good candidate for neural tissue engineering.

2.1.3 Dextran—A modified precursor of the enzymatically biodegradable dextran (Dex) was formed by reaction with maleic anhydride (MA) and the Dex-MA polysaccharide was given thermoresponsive properties by photocrosslinking it with NiPAAm. The resulting hydrogel was partially biodegradable and exhibited a higher LCST than pNiPAAm due to the hydrophilic and biodegradable nature of Dex-MA. Additionally, the carboxylic end groups of Dex-MA render the hydrogel pH sensitive [30]. Another approach based on a dextran polysaccharide was reported by Huang et al [31]. A dextran macromer containing oligolactate and 2-hydroxyethyl methacrylate units (Dex-lactate-HEMA), which has hydrolytically degradable blocks, was copolymerized with NiPAAm. This hydrogel showed an LCST close to that of pNiPAAm (approximately 32°C). Its swelling and degradation in phosphate buffered saline (PBS) were studied at 25 and 37°C. At 25°C, which is below the LCST, the hydrogels had disintegrated within two weeks, with the rate of dissolution depending on their composition. At 37°C however, the degradation was much slower due to increased hydrophobic effects. Interestingly, when the hydrogel was tested for drug delivery, it was shown that a low molecular weight drug (methylene blue) was released slower at 25°C than at 37°C, whereas the opposite was observed for a high molecular weight substance (bovine serum albumine –BSA) (figure 3). The authors concluded that the drug release profile depends on a number of factors, as the temperature, the swelling and degradation characteristics of the hydrogel, as well as the interactions of the drug and the hydrogel macromolecules.

2.1.4 Xyloglucan—Xyloglucan is a cytocompatible polysaccharide and has exhibited thermally responsive behavior when more than 35% of its galactose residues are removed [32]. Xyloglucan gels have been used as a drug delivery vehicle for various applications [19], however there are not many data on the rheological and morphological characteristics of these hydrogels. Nisbet et al. [33] have examined the gelation properties of xyloglucan hydrogels as well as their morphology under physiological conditions. The gelation process seemed to be influenced by the presence of ions in PBS as compared to deionized water. As to the optimum concentration, it was found that 3% (wt.) xyloglucan in aqueous media possess an elastic modulus that is significantly higher than other natural or synthetic hydrogels. Moreover, this concentration yielded a gel that could be freeze-dried and examined with scanning electron microscopy. The images showed a macroporous, interconnected, three-dimensional network.

2.2 Proteins

2.2.1 Gelatin—Gelatin is another biopolymer with thermoreversible properties. At temperatures below 25°C, an aqueous gelatin solution solidifies due to the formation of triple helices and a rigid three-dimensional network. When the temperature is raised above approximately 30°C, the conformation changes from a helix to the more flexible coil, rendering the gel liquid again [17]. As the opposite thermal behavior is desired for biomedical applications, researchers have combined gelatin with other polymers, which

show thermal gelation close to body temperature. Gelatin has the advantage of allowing for easy modification on the amino acid level; moreover, it is biodegradable and biocompatible [34]. A binary-component hydrogel composed of gelatin and monomethoxy poly(ethylene glycol)-poly(D,L-lactide) (mPEG-DLLA) block copolymers was synthesized by [34]. For most compositions of gelatin and mPEG-DLLA, the hydrogel was shown to flow at 37°C and gel at room temperature, however a 100 mg/mL gelatin solution underwent fast gelation at 37°C when mixed with 30% wt. mPEG-DLLA. Different hydrogel compositions were also examined for drug release kinetics with gentamycin sulfate as the model drug. At room temperature, five days or longer was necessary for 50% drug release, and the release lasted up to 40 days. At 37°C, gelatin-mPEG-DLLA showed an even slower release profile, however after one week the release was no longer detectable due to degradation of the hydrogel matrices.

Ohya and Matsuda [35] have grafted gelatin with NiPAAm in an effort to produce a thermoresponsive extracellular matrix analogue. Aqueous solutions showed a sol-gel transition at physiological temperature when the weight ratio of pNiPAAm to gelatin chains was higher than 5.8. Smooth muscle cells were suspended in medium solutions of pNiPAAm/gelatin and subsequently incubated at 37°C. It was shown that a low hydrogel concentration (5% w/v) and a high pNiPAAm to gelatin ratio (P/G) supported the highest cell proliferation and extracellular matrix production. The authors suggested that this was due to increased hydrophobicity caused by higher pNiPAAm ratios, which would lead to the formation of large aggregates. As a result, a higher porosity with larger pore size occurs, which comprises a favorable cell environment (figure 4).

Another protein-based hydrogel was proposed by Gil et al. [36]. Gelatin was blended with silk fibroin to yield a thermoresponsive gel, which was stabilized at 37°C by the presence of β crystals of silk fibroin. The swelling profile at temperatures below and above the helix-to-coil transformation of gelatin was evaluated, as well as the protein release from the matrices. The gel showed a higher swelling at physiological temperatures as compared to 20°C, but also higher mass loss due to dissolution and release of gelatin.

3. *N*-Isopropylacrylamide-based systems

Hydrogels based on poly(*N*-isopropylacrylamide) (pNiPAAm) and its copolymers belong to the most intensively investigated thermoreversible systems. Recent developments on pNiPAAm-based hydrogels include their use for drug delivery [12, 13, 37, 38], cell encapsulation and delivery [39, 40] and cell culture surfaces [41]. Poly(*N*-isopropylacrylamide) is non-biodegradable and exhibits a sharp phase transition, with an LCST at about 32°C in pure water [37, 42]. Below the LCST, pNiPAAm assumes a flexible, extended coil conformation in aqueous solutions. At the LCST, it becomes hydrophobic and the polymer chains seem to collapse prior to aggregation in globular structures [14, 43]. Copolymerization of NiPAAm with a more hydrophilic monomer increases the overall hydrophilicity of the polymer, and the stronger polymer-water interactions lead to an increase in the LCST. Likewise, copolymerization with a more hydrophobic monomer results in a lower LCST than pNiPAAm [44]. Moreover, the phase transition temperature is influenced by the presence of salts [45] and pH to a certain extent [42, 45].

Coughlan and colleagues [38] evaluated the swelling and release profile of crosslinked pNiPAAm hydrogels as a function of the physicochemical properties of the loaded drugs. Dried hydrogel discs were loaded by sorption of a drug solution, the solvent was removed and the hydrogels were allowed to swell in a buffer solution. Hydrogel swelling was decreased in the presence of hydrophobic drugs and the opposite effect was observed for hydrophilic drugs. In temperatures above the LCST, the system showed contraction and

deswelling, and a solubility-dependent drug pulse release was shown for hydrophobic drugs, whereas hydrophilic drugs showed a molecular weight-dependent drug pulse. The authors suggested that drug properties such as solubility, size and chemical nature should be considered when a thermosensitive hydrogel as pNiPAAm is chosen as a delivery vehicle.

Copolymers of NiPAAM have also been popular in an attempt to yield hydrogels with thermal responsiveness and improved properties. The hydrogel potential of NiPAAM copolymers with acrylic (AA) [12, 39] and propylacrylic acid (PAA) [13] was examined. The thermoreversible p(NiPAAm-*co*-AA) hydrogel was tested as a cell and drug delivery vehicle. Chondrocytes, dexamethasone and ascorbate as differentiation factors as well as transforming growth factor β3 (TGF-β3) were encapsulated in the hydrogel and were implanted subcutaneously in mice. The chondrogenic factors were provided in order to hinder chondrocyte de-differentiation *in vivo.* After 8 weeks, significant collagen II expression as well as proteoglycan and polysaccharide production was evident, indicating that the cells had preserved their phenotype. This hydrogel in combination with the differentiation and growth factors holds promise for cartilage tissue engineering [39]. Liu [12] et al have synthesized p(NiPAAm-*co*-AA) and polymerized it with ethyl acrylate (EA) using the interpenetrating polymer network (IPN) technology. An interpenetrating polymer network is formed by hydrophilic and hydrophobic networks that are only physically interconnected, without any chemical bonding, so that individual components retain their original properties. The same group [46] had found that IPN structures are effective amphiphilic drug carriers. A pH dependence on the swelling ratio of p(NiPAAm-*co*-AA) as well as of interpenetrating polymer network with ethyl acrylate (p(NiPAAm-*co*-AA)/pEA IPN) was observed at 37°C. This was attributed to the presence of the carboxyl group on the acrylic acid. Swelling was lower on the IPNs due to the hydrophobic pEA. The drug release kinetics of both hydrogels were evaluated using daidzein as a model drug. P(NiPAAm-*co*-AA) showed an initial burst release, which was not observed on p(pNiPAAm-*co*-AA)/pEA IPN. It was concluded that the pEA chains in the IPN structure had a favorable effect in maintaining a slower and more stable release profile [12]. Also, copolymers of NiPAAm with propylacrylic acid (PAA) show a temperature and pH-sensitive behavior. Yin et al [13] synthesized copolymers by a reversible addition fragmentation transfer (RAFT) method, using different NiPAAm and PAA ratios. They showed that even small changes in pH can have a big effect on the LCST of the hydrogel. This feature can be useful for applications such as drug delivery, where physiological temperature and local pH differences can both act as stimuli, and for molecular switching over a desired pH range.

An interesting approach to a combination of stimuli-responsive attributes was recently proposed by Xu et al. [47] in the form of a triblock copolymer hydrogel. A poly((2-dimethyl amino)ethyl methacrylate-*co*-2-hydroxyethyl methacrylate)-*b*-poly(*N*-isopropylacrylamide)-*b*-poly((2-dimethyl amino)ethyl methacrylate-*co*-2-hydroxyethyl methacrylate) or p(DMAEMA-*co*-HEMA)-*b*-p(NiPAAm)-*b*-p(DMAEMA-*co*-HEMA) copolymer was synthesized by atom transfer radical polymerizations (ATPR). The hydroxyl groups on HEMA allowed for chemical crosslinking with glutaraldehyde. The hydrogel showed combined characteristics of its building blocks: Its temperature-responsive behavior was attributed to pNiPAAm and the pH-sensitivity to pDMAEMA. Our group [40] has synthesized thermogelling macromers for fabrication of a hydrogel for orthopedic tissue engineering applications. The aim was to yield a gel with better mechanical properties than most hydrophilic injectable hydrogels. This was accomplished by incorporating a hydrophobic domain that provides cohesive interactions as well as functional groups for chemical crosslinking. A random copolymer of pentaerythritol monostearate diacrylate (PEDAS), *N*-isopropylacrylamide (NiPAAm), acrylamide (AAm) and 2-hydroxyethyl acrylate (HEA) was synthesized. PEDAS contains a lipophilic side chain, and AAm and HEA can modulate hydrophilicity and add groups for subsequent acrylation and

crosslinking. The thermal gelation is attributed to the NiPAAm block. Our studies so far have shown that the macromers possess a thermoreversible behavior. Future work is directed into further development and characterization of the hydrogel and examination of its potential in bone regeneration.

Another issue that has to be addressed is biodegradability. Many homo-and copolymers of NiPAAm are not biodegradable [48], a fact that may prove problematic for some biomedical engineering applications. Nakayama and colleagues [37] have prepared thermally responsive, biodegradable polymeric micelles for controlled drug release. A hydrophobic block in the micelles was used to incorporate water-insoluble drugs. By combining a poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) (p(NiPAAm-*co*-DMAAm)) block, which has an LCST around 40°C, with poly(D,L-lactide), poly(ε-caprolactone) or poly(D,L-lactide-*co*-ε-caprolactone), which are all biodegradable and hydrophobic, the group was able to fabricate polymeric micelles with controlled dimensions and phase transition temperatures. Below the LCST, the thermoresponsive block forms the outer shell of the micelle, but upon temperature increase above the LCST, the block becomes increasingly hydrophobic and shrinks. In the case of p(NiPAAm-*co*-DMAAm)-*b*-p(D,L-lactide-*co*-ε-caprolactone) diblock copolymer, temperature increase above the LCST proved to facilitate drug release (figure 5).

Polymers based on pNiPAAm have found applications in another field crucial to biomedical scientists: Their thermoresponsive behavior has been proven useful in cell culture substrates. By introducing a pNiPAAm layer on tissue culture plates, the hydrophilicity of the substrate can be modulated with temperature switch. It is well known that most cells preferentially adhere to hydrophobic surfaces. Above its LCST (32°C), pNiPAAm shows a hydrophobic behavior. It represents therefore a suitable surface for cell attachment and proliferation at physiological temperature. By lowering the temperature below the LCST, the culture surface becomes hydrophilic and the cells automatically detach [49]. This cell recovery technique is a good alternative to the conventional, but often damaging, enzymatic or mechanical detachment methods. Recently, Hatakeyama et al. [41] have been producing bioactive, thermoresponsive cell culture surfaces by immobilizing the cell adhesive peptide RGDS and the growth factor insulin on a NiPAAm-copolymer. *N*-Isopropylacrylamide was copolymerized with its analogue 2-carboxyisopropylacrylamide and the polymer was grafted onto polystyrene tissue culture dishes, followed by RGDS and insulin immobilization. They found that these factors increase cell adhesion and proliferation, reducing therefore culture time. When the temperature was brought to 20°C, the cells could be easily recovered as contiguous tissue monolayers.

4. PEO/PPO-based systems

Triblock copolymers poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO), known also as Pluronics® or Poloxamers, are another important group of synthetic polymers with a thermoreversible behavior in aqueous solutions. By adjusting the composition, the molecular weight and the concentration, this reversible gelation can occur at physiological temperature and pH [50]. The polymers owe their amphiphilic structure to the hydrophilic ethylene oxide and the hydrophobic propylene oxide. The gelation mechanism of PEO/PPO block copolymers in aqueous solutions has been a topic of extensive investigation, and a possible explanation to this phenomenon could be given by the changes in micellar properties as a function of both concentration and temperature. Amphiphilic block copolymer molecules can self assemble into micelles in aqueous solutions. Above a certain concentration, termed as the critical micelle concentration (CMC), the polymer molecules, which were previously in solution, aggregate and form micelles. Members of the Pluronics® family which are used for drug delivery exhibit a

CMC of 1 μM to 1 mM at 37°C. Moreover, the micelle formation has strong temperature dependence. Below a certain temperature, termed as the critical micelle temperature (CMT), both ethylene and propylene oxide blocks are hydrated and poly(propylene oxide) is relatively soluble in water. With temperature increase, poly(propylene oxide) chains become less soluble, resulting in micelle formation [16].

As Pluronics® are commercially available in a range of molecular weights, composition ratios and forms, it would be useful to mention the nomenclature rules for these copolymers. The letter in the notation stands for liquid (L), paste (P) or flakes (F), whereas the first two numbers indicate the molecular weight of the PPO block and the last number the weight fraction of the PEO block. For example, the commonly used in biomedical applications F127 has a weight percentage of 70% PEO and a molecular weight of PPO around 4000 [50].

Over the past years, these copolymers have been extensively used in applications such as drug and gene delivery [16], inhibition of tissue adhesion [51, 52] and burn wound covering [53, 54]. Newer advances in gene delivery are summarized elsewhere [55]. Pluronics® represent a bio-inert environment, attributed by the hydrophilicity and flexibility of PEO chains [56]. Therefore, most cells do not grow on these polymers, which have been used as tissue adhesion barriers. However, it was shown that Pluronics® can be a good substrate for hematopoietic stem cells, supporting their culture and preservation more than conventional tissue culture dishes [57, 58]. Recently, the use of Pluronic® F127 (synonymous to poloxamer 407) was reported for tissue engineering applications. This polymer has been found to have a rapid gelation at 37°C (after one-minute incubation, 30% solution in cell culture medium) [59]. F127 was evaluated as a scaffold for lung tissue engineering, showing promising results on tissue growth with low inflammatory response [60]. Weinand and colleagues [59] tested a β-tricalcium phosphate (β-TCP) scaffold, using a F127 hydrogel to facilitate cell delivery and distribution for an *in vitro* study aiming at bone regeneration. They reported that F127 was no longer present in the channels of the β-TCP scaffold after one week in culture and seemed to have degraded. Bone tissue growth was only weakly induced, and the constructs showed lower stiffness than other hydrogel (fibrin, collagen I) composites evaluated.

In general, Pluronic® F127 has been found to have inadequate mechanical integrity which makes them inappropriate for certain biomedical applications. The hydrogels show a low viscosity, which has as consequences poor mechanical strength, high permeability and limited stability with quick dissolution [61, 62]. Cohn and colleagues proposed two new mechanisms to create copolymers based on Pluronic® F127 with improved mechanical properties. In both cases they relied on the principle of a multiblock backbone with the addition of covalently bound repeating units. This way, the macromolecular structure and orientation could be controlled. The first involved the polymerization of F127, with hexamethylene diisocyanate as a chain extender, forming poly(ether-urethanes). The second relied on the covalent binding of poly(ethylene glycol) and poly(propylene glycol), which as such do not possess thermogelling properties at physiological temperatures, using phosgene as a coupler and forming poly(ether-carbonates). Both newly synthesized polymers exhibited significantly higher viscosities than F127 at 37°C, and the poly(ether-urethanes) displayed much slower drug release kinetics than the original polymer [61]. The group developed the idea of thermoresponsive PEO/PPO polymers with improved mechanical behavior further in the next years. Their strategies included i) introduction of end groups that would allow for *in situ* chemical crosslinking after thermal gelation, such as carbon-carbon double bonds [63], ethoxysilane groups [62, 64] and methacrylate groups [64] ii) synthesis of poly(ethylene oxide) and poly(propylene oxide) block copolymers using diacyl chloride as a coupling agent [65] iii) synthesis of PEO/PPO copolymers with incorporation of ε-

caprolactone [65, 66] or lactide [66] oligoester segments prior to chain extension. The latter approach yielded biodegradable hydrogels due to hydrolytic cleavage of the ester bonds.

The need for more stable hydrogels was identified also by Cellesi et al. [67–69]. Their approach mimicked the natural thermal gelation of alginate by relying on the occurrence of a physical mechanism, attributed by the thermosensitive nature of Pluronics®, followed by an irreversible chemical mechanism, due to covalent crosslinking by the reaction of groups at the termini of the copolymer. They named their gelation approach “tandem process” due to the cooperative action of both mechanisms (figure 6).

Pluronic® polymers were functionalized with acrylic moieties and thiols at their end groups and were subsequently gelled at 37°C, where a Michael-type addition took place and allowed for a slower chemical curing. It was found that these polymers were biocompatible, and so was their gelation process, which can be performed at physiological temperature and pH, allowing for encapsulation of sensitive drugs and cells [67]. In order to limit steric hindrance phenomena, a similar method was followed with Tetronic® polymers, which are thermosensitive tetra-armed Pluronic® analogues [68]. By adjusting the molecular weight of the precursors and the functionalization (therefore also the crosslinking density), the final mechanical and transport properties of the “tandem” polymers can be controlled [68, 69]. Moreover, the “tandem” method allows for easy processing of the polymers, for example into spherical beads and hollow capsules [69].

5. Other synthetic polymers

5.1 PEG/Biodegradable polyester copolymers

The copolymerization of hydrophilic, biocompatible poly(ethylene glycol) (PEG) with biodegradable and biocompatible polyesters has yielded some interesting hydrogel systems. Thermoresponsive properties were given by the appropriate adjustment of the hydrophobic polyester block and the PEG block length.

In 1997, Jeong [70] and colleagues reported the synthesis of injectable poly(ethylene glycol)-*b*-poly(D,L-lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol) (PEG-PLGA-PEG) triblock copolymers. These polymers were biocompatible, biodegradable and exhibited a sol-gel transition. The use of high molecular weight-PLGA combined with low molecular weight-PEG resulted in a hydrogel with quick gelation at physiological temperature. The combination of hydrophobic/hydrophilic units created a surfactant behavior of the polymers in water, facilitating thus also the solubilization of hydrophobic drugs. *In vivo* studies showed sufficient mechanical properties and integrity for longer than a month [71]. More recently, Chen et al. [72] developed a triblock PLGA-PEG-PLGA-based system for the controlled release of testosterone. Testosterone is water-insoluble and so far, its delivery systems included patches, creams, gels, injectables and implants [73]. A slower *in vitro* release of testosterone was observed for copolymers with longer PLGA blocks, possibly due to the slower degradation of these hydrophobic units. The thermosensitive polymers showed a controlled, linear release for a period of three months.

Another recent approach towards a thermoresponsive system involved the synthesis of a multiblock copolymer with a biodegradable polyester. Alternating multiblock poly(ethylene glycol)/poly(L-lactic acid) (PEG/PLLA) copolymers were produced. It was shown that sol-to-gel transition was depending on both the total molecular weight (MW) and the MW of each building block. *In vitro* and *in vivo* gelation studies determined that a copolymer with a total MW of 6700 daltons and 600/1300 (MW of PEG/PLLA blocks respectively) holds potential as an injectable carrier for biomedical applications in terms of transition temperature and modulus at 37°C (figure 7) [74].

Our group has proposed the combination of methoxy poly(ethylene glycol) (mPEG) with poly(propylene fumarate) (PPF) and the synthesis of a mPEG-PPF-mPEG triblock copolymer [75]. Copolymers exhibited an LCST depending on the molecular weight of mPEG, and it was shown that their LCST was strongly influenced by the presence of salts. Moreover, the presence of the fumarate double bonds on PPF can allow for chemical crosslinking, enhancing thus the stability of the hydrogels. Recently, this hydrogel was evaluated for articular cartilage tissue engineering [76]. Chondrocytes were encapsulated in the hydrogel at 37°C and subsequently tested for their phenotypic characteristics. It was found that chondrocytes cultured in PEG/PPF hydrogels proliferated and produced significant levels of proteoglycans and collagen type II, which are both markers of the chondrocytic phenotype. When compared to cells cultured in agarose and alginate hydrogels, two materials widely studied for chondrocyte delivery, proliferation levels in PEG/PPF were similar, however proteoglycan and collagen production was lower. Supplement of the bone morphogenic protein 7 in PEG/PPF hydrogels was also shown to increase chondrocyte proliferation, but not proteoglycan synthesis.

5.2 Poly(organophosphazenes)

Current advances on poly(organophosphazenes) include their use as drug [77, 78] and cell [79] delivery systems. Poly(organophosphazenes) grafted with mPEG and amino acid esters were reported as a new class of biodegradable and thermosensitive polymers in 1999 [48]. Sohn and colleagues [80] developed a correlation for the LCST of these polymers as a function of their molecular structure, which comprises hydrophilic (PEG) and hydrophobic (amino acid esters) side groups. The polymers showed a sustained release profile for both hydrophobic [77] as well as hydrophilic [78] drugs for over three and two weeks respectively. Also their use as cell carriers holds promise, as shown recently. Hepatocytes cultured in poly(organophosphazene) hydrogels were able to maintain good viability and liver-specific activity for a period of four weeks [79].

6. Conclusions

Research in the area of thermoresponsive polymers for drug and gene delivery as well as for tissue adhesion prevention and wound covering has been well established in the past years. More recently, hydrogels exhibiting a thermosensitive sol-gel behavior have been reported as cell carriers for tissue regeneration. Typically, aqueous solutions of hydrogels used in biomedical applications are liquid at ambient temperature and gel at 37°C. Poly(*N*-isopropylacrylamide) and its copolymers with other synthetic or natural polymers is one of the most investigated thermoresponsive systems. By the appropriate copolymerization, intrinsic drawbacks of pNiPAAm like its non-biodegradability and mechanical properties may be improved. Moreover, researchers have achieved to resolve the instability problems of another popular system, Pluronics®. It is important to recognize the properties of the system(s) the hydrogel will be in contact with (such as hydrophilic or hydrophobic drugs, cells) and also the way and locus of administration in order to optimize the result. Each application has different requirements, and it is possible to tailor the hydrogel properties to match a specific use.

Acknowledgments

Research in the area of synthetic polymers for biomedical applications has been funded by the National Institutes of Health (Grant numbers: R01 DE15164 and R01 AR48756).

References

1. Peppas, NA.; Mikos, AG. Preparation methods and structure of hydrogels. In: Peppas, NA., editor. Hydrogels in Medicine and Pharmacy. Vol. 1. CRC Press; Boca Raton, Florida: 1986. p. 1-25.
2. Peppas, NA. Biomaterials Science, An Introduction to Materials in Medicine. Academic Press; San Diego, California: 1996. Hydrogels; p. 60-64.
3. Hoffman AS. Hydrogels for biomedical applications. Adv Drug Deliv Rev. 2002; 43:3–12. [PubMed: 11755703]
4. Burkoth AK, Anseth KS. A review of photocrosslinked polyanhydrides: in situ forming degradable networks. Biomaterials. 2000; 21:2395–2404. [PubMed: 11055287]
5. Burdick JA, Frankel D, Dernell WS, Anseth KS. An initial investigation of photocurable three-dimensional lactic acid based scaffolds in a critical-sized cranial defect. Biomaterials. 2003; 24:1613–1620. [PubMed: 12559821]
6. Ossipov DA, Hilborn J. Poly(vinyl alcohol)-based hydrogels formed by “click chemistry”. Macromolecules. 2006; 39:1709–1718.
7. Shu XZ, Ahmad S, Liu Y, Prestwich GD. Synthesis and evaluation of injectable, *in situ* crosslinkable synthetic extracellular matrices for tissue engineering. J Biomed Mater Res. 2006; 79:902–912.
8. Kuo CK, Ma PX. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties. Biomaterials. 2001; 22:511–521. [PubMed: 11219714]
9. Peppas NA. Physiologically responsive gels. J Bioact Compat Polym. 1991; 6:241–246.
10. Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm. 2000; 50:27–46. [PubMed: 10840191]
11. Li L, Shan H, Yue CY, Lam YC, Tam KC, Hu X. Thermally induced association and dissociation of methylcellulose in aqueous solutions. Langmuir. 2002; 18:7291–7298.
12. Liu YY, Shao YH, Lü J. Preparation properties and controlled release behaviors of pH-induced thermosensitive amphiphilic gels. Biomaterials. 2006; 27:4016–4024. [PubMed: 16563494]
13. Yin X, Hoffman AS, Stayton PS. Poly(*N*-isopropylacrylamide-*co*-propylacrylic acid) copolymers that respond sharply to temperature and pH. Biomacromolecules. 2006; 7:1381–1385. [PubMed: 16677016]
14. Schild HG. Poly(*N*-isopropylacrylamide): experiment, theory and application. Prog Polym Sci. 1992; 17:163–249.
15. Southall NT, Dill KA, Haymet ADJ. A view of the hydrophobic effect. J Phys Chem B. 2002; 106:521–533.
16. Kabanov AV, Batrakova EV, Alakhov VY. Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery. J Control Release. 2002; 82:189–212. [PubMed: 12175737]
17. Joly-Duhamel C, Hellio D, Djabourov M. All gelatin networks: 1. Biodiversity and physical chemistry. Langmuir. 2002; 18:7208–7217.
18. Jeong B, Kim SW, Bae YH. Thermosensitive sol-gel reversible hydrogels. Adv Drug Deliv Rev. 2002; 54:37–51. [PubMed: 11755705]
19. Ruel-Gariepy E, Leroux JC. In situ forming hydrogels-review of temperature-sensitive systems. Eur J Pharm Biopharm. 2004; 58:409–426. [PubMed: 15296964]
20. Takahashi M, Shimazaki M, Yamamoto J. Thermoreversible gelation and phase separation in aqueous methyl cellulose solutions. J Polym Sci B. 2001; 39:91–100.
21. Liu W, Zhang B, Lu WW, Li X, Zhu D, De Yao K, Wang Q, Zhao C, Wang C. A rapid temperature-responsive sol-gel reversible poly(*N*-isopropylacrylamide)-*g*-methylcellulose copolymer hydrogel. Biomaterials. 2004; 25:3005–3012. [PubMed: 14967533]
22. Stabenfeldt SE, Garcia AJ, LaPlaca MC. Thermoreversible laminin-functionalized hydrogel for neural tissue engineering. J Biomed Mater Res. 2006; 77A:718–725.

23. Tate MC, Shear DA, Hoffman SW, Stein DG, LaPlaca MC. Biocompatibility of methylcellulose-based constructs designed for intracerebral gelation following experimental traumatic brain injury. Biomaterials. 2001; 22:1113–1123. [PubMed: 11352091]
24. Bhattarai N, Matsen FA, Zhang M. PEG-grafted chitosan as an injectable thermoreversible hydrogel. Macromol Biosci. 2005; 5:107–111. [PubMed: 15719428]
25. Bhattarai N, Ramay HR, Gunn J, Matsen FA, Zhang M. PEG-grafted chitosan as an injectable thermosensitive hydrogel for sustained protein release. J Control Release. 2005; 103:609–624. [PubMed: 15820408]
26. Cho JH, Kim SH, Park KD, Jung MC, Yang WI, Han SW, Noh JY, Lee JW. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(*N*-isopropylacrylamide) and water-soluble chitosan copolymer. Biomaterials. 2004; 25:5743–5751. [PubMed: 15147820]
27. Dang JM, Sun DDN, Shin-Ya Y, Sieber AN, Kostuik JP, Leong KW. Temperature-responsive hydroxylbutyl chitosan for the culture of mesenchymal stem cells and intervertebral stem cells. Biomaterials. 2006; 27:406–418. [PubMed: 16115680]
28. Chenite A, Chaput C, Wang D, Combes C, Buschmann MD, Hoemann CD, Leroux JC, Atkinson BL, Binette F, Selmani A. Novel injectable neutral solutions of chitosan form biodegradable gels in situ. Biomaterials. 2000; 21:2155–2161. [PubMed: 10985488]
29. Crompton KE, Goud JD, Bellamkonda RV, Gengenbach TR, Finkelstein DI, Horne MK, Forsythe JS. Polylysine-functionalised thermoresponsive chitosan hydrogel for neural tissue engineering. Biomaterials. 200610.1016/j.biomaterials.2006.08.044
30. Zhang X, Wu D, Chu CC. Synthesis and characterization of partially biodegradable, temperature and pH sensitive Dex-MA/PNIPAAm hydrogels. Biomaterials. 2004; 25:4719–4730. [PubMed: 15120518]
31. Huang, Lowe TL. Biodegradable thermoresponsive hydrogels for aqueous encapsulation and controlled release of hydrophilic model drugs. Biomacromolecules. 2005; 6:2131–2139. [PubMed: 16004455]
32. Shirakawa, Yamotoya K, Nishinari K. Tailoring of xyloglucan properties using an enzyme. Food Hydrocoll. 1998; 12:25–28.
33. Nisbet DR, Crompton KE, Hamilton SD, Shirakawa S, Prankerd RJ, Finkelstein DI, Horne MK, Forsythe JS. Morphology and gelation of thermosensitive xyloglucan hydrogels. Biophys Chem. 2006; 121:14–20. [PubMed: 16406645]
34. Yang H, Kao WJ. Thermoresponsive gelatin/monomethoxy poly(ethylene glycol)-poly(D,L-lactide) hydrogels: Formulation, characterization and antibacterial drug delivery. Pharm Res. 2006; 23:205–214. [PubMed: 16270162]
35. Ohya S, Matsuda T. Poly (*N*-isopropylacrylamide) (PNIPAM)-grafted gelatin as thermoresponsive three-dimensional artificial extracellular matrix: molecular and formulation parameters vs. cell proliferation potential. J Biomater Sci Polym Ed. 2005; 16:809–827. [PubMed: 16128290]
36. Gil ES, Frankowski DJ, Spontak RJ, Hudson SM. Swelling behavior and morphological evolution of mixed gelatin/silk fibroin hydrogels. Biomacromolecules. 2005; 6:3079–3087. [PubMed: 16283730]
37. Nakayama M, Okano T, Miyazaki T, Kohori F, Sakai K, Yokoyama M. Molecular design of biodegradable polymeric micelles for temperature-responsive drug release. J Control Release. 200610.1016/j.conrel.2006.07.007
38. Coughlan DC, Quilty FP, Corrigan OI. Effect of drug physichochemical properties on swelling/deswelling kinetics and pulsatile drug release from thermoresponsive poly(*N*-isopropylacrylamide) hydrogels. J Control Release. 2006; 98:97–114. [PubMed: 15245893]
39. Na K, Park JH, Kim SW, Sun BK, Woo DG, Chung HM, Park KH. Delivery of dexamethasone, ascorbate, and growth factor (TGF β-3) in thermo-reversible hydrogel constructs embedded with rabbit chondrocytes. Biomaterials. 2006; 27:5951–5957. [PubMed: 16949668]
40. Hacker, MC.; Ma, BB.; Kretlow, JD.; Mikos, AG. Novel macromers for the fabrication of injectable, calcium-binding hydrogels. Transactions of the 31st Annual Meeting of the Society for Biomaterials; Pittsburgh, USA. April 26–29 2006;

41. Hatakeyama, Kikuchi A, Yamato M, Okano T. Bio-functionalized thermoresponsive interfaces facilitating cell adhesion and proliferation. Biomaterials. 2006; 27:5069–5078. [PubMed: 16782188]

42. Pei Y, Chen J, Yang L, Shi L, Tao Q, Hui B, Li J. The effect of pH on the LCST of poly(*N*-isopropylacrylamide) and poly(*N*-isopropylacrylamide-co-acrylic acid). J Biomater Sci Polym Ed. 2004; 15:585–594. [PubMed: 15264660]

43. Zhang XZ, Yang YY, Chung TS, Ma KX. Preparation and characterization of fast response macroporous poly(*N*-isopropylacrylamide) hydrogels. Langmuir. 2001; 17:6094–6099.

44. Feil H, Bae YH, Feijen J, Kim SW. Effect of comonomer hydrophilicity and ionization on the lower critical solution temperature of *N*-isopropylacrylamide copolymers. Macromolecules. 1993; 26:2496–2500.

45. Liu XM, Wang LS, Wang L, Huang J, He C. The effect of salt and pH on the phase-transition behaviors of temperature-sensitive copolymers based on *N*-isopropylacrylamide. Biomaterials. 2004; 25:5659–5666. [PubMed: 15159082]

46. Liu YY, Fan XD, Wei BR, Si QF, Chen WX, Sun L. pH-responsive amphiphilic hydrogel networks with IPN structure: a strategy for controlled drug release. Int J Pharm. 2006; 308:205–209. [PubMed: 16321489]

47. Xu FJ, Kang ET, Neoh KG. pH- and temperature-responsive hydrogels from crosslinked triblock copolymers prepared via consecutive atom transfer radical polymerization. Biomaterials. 2006; 27:2787–2797. [PubMed: 16442613]

48. Song SC, Lee SB, Jin JI, Sohn YS. A new class of biodegradable thermosensitive polymers. I. Synthesis and characterization of poly(organophosphazenes) with methoxy-poly(ethylene glycol) and amino acid esters as side groups. Macromolecules. 1999; 32:2188–2193.

49. Okano T, Tamada N, Sakai H, Sakurai Y. A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly(*N*-isopropylacrylamide). J Biomed Mater Res. 1993; 27:1243–1251. [PubMed: 8245039]

50. Alexandridis P, Hatton TA. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and interfaces: thermodynamics, structure, dynamics and modeling. Colloids Surf A. 1995; 96:1–46.

51. Steinleitner A, Lambert H, Kazensky C, Cantor B. Poloxamer 407 as an intraperitoneal barrier material fort he prevention of postsurgical adhesion formation and reformation in rodent models for reproductive surgery. Obstet Gynecol. 1991; 77:48–52. [PubMed: 1984226]

52. Ahmed F, Alexandridis P, Shankaran H, Neelamegham S. T The ability of poloxamers to inhibit platelet aggregation depends on their physicochemical properties. Thromb Haemost. 2001; 86:1532–1539. [PubMed: 11776324]

53. Schmolka IR. Artificial skin. I. Preparation and properties of pluronic F-127 gels for treatment of burns. J Biomed Mater Res. 1972; 6:571–582. [PubMed: 4642986]

54. Nalbandian RM, Henry RL, Balko KW, Adams DV, Neuman NR. Pluronic F-127 gel preparation as an artificial skin in the treatment of third-degree burns in pigs. J Biomed Mater Res. 1987; 21:1135–1148. [PubMed: 3667639]

55. Kabanov A, Zhu J, Alakhov V. Pluronic Block Copolymers for Gene Delivery. Adv Genet. 2005; 53:231–261. [PubMed: 16240996]

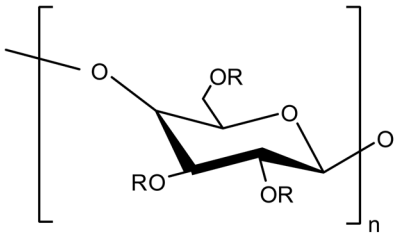
56. Higuchi A, Sugiyama K, Yoon BO, Sakurai M, Hara M, Sumita M, Sugawara S, Shirai T. Serum protein adsorption and platelet adhesion on pluronic-adsorbed polysulfone membranes. Biomaterials. 2003; 24:3235–3245. [PubMed: 12763451]

57. Higuchi A, Aoki N, Yamamoto T, Gomei Y, Egashira S, Matsuoka Y, Miyazaki T, Fukushima H, Lee YM, Jyujyoji S, Natori SH. Bio-inert surface of Pluronic-immobilized flask for preservation of haematopoietic stem cells. Biomacromolecules. 2006; 7:1083–1089. [PubMed: 16602724]

58. Higuchi A, Aoki N, Yamamoto T, Miyazaki T, Fukushima H, Tak TM, Jyujyoji S, Egashira S, Matsuoka Y, Natori SH. Temperature-induced cell detachment on immobilized pluronic surface. J Biomed Mater Res A. 2006; 79:380–392. [PubMed: 16883586]

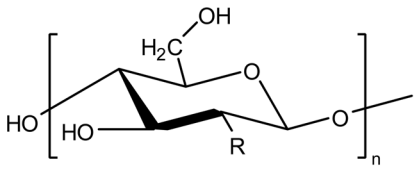
59. Weinand C, Pomerantseva I, Neville CM, Gupta R, Weinberg E, Madisch I, Shapiro F, Abukawa H, Troulis MJ, Vacanti JP. Hydrogel-β-TCP scaffolds and stem cells for tissue engineering bone. Bone. 2006; 38:555–563. [PubMed: 16376162]

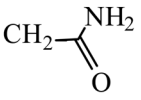
60. Cortiella J, Nichols JE, Kojima K, Bonassar LJ, Dargon P, Roy AK, Vacant MP, Niles JA, Vacanti CA. Tissue-engineered lung: An *in vivo* and *in vitro* comparison of polyglycolic acid and Pluronic F-127 hydrogels/somatic lung progenitor cell constructs to support tissue growth. Tissue Eng. 2006; 12:1213–1225. [PubMed: 16771635]
61. Cohn D, Sosnik A, Levy A. Improved reverse thermo-responsive polymeric systems. Biomaterials. 2003; 24:3707–3714. [PubMed: 12818542]
62. Sosnik A, Cohn D. Ethoxysilane-capped PEO-PPO-PEO triblocks: a new family of reverse thermo-responsive polymers. Biomaterials. 2004; 25:2851–2858. [PubMed: 14962563]
63. Sosnik A, Cohn D, San Roman J, Abraham GA. Crosslinkable PEO-PPO-PEO-based reverse thermo-responsive gels as potentially injectable materials. J Biomater Sci Polym Ed. 2003; 14:227–239. [PubMed: 12713096]
64. Cohn D, Sosnik A, Garty S. Smart hydrogels for *in situ* generated implants. Biomacromolecules. 2005; 6:1168–1175. [PubMed: 15877330]
65. Sosnik A, Cohn D. Reverse thermo-responsive poly(ethylene oxide) and poly (propylene oxide) multiblock copolymers. Biomaterials. 2005; 26:349–357. [PubMed: 15275809]
66. Cohn D, Lando G, Sosnik A, Garty S, Levi A. PEO-PPO-PEO-based poly (ether ester urethane)s as degradable reverse thermo-responsive multiblock copolymers. Biomaterials. 2006; 27:1718–1727. [PubMed: 16310849]
67. Cellesi F, Tirelli N, Hubbell JA. Materials for cell encapsulation via a new tandem approach combining reverse thermal gelation and covalent crosslinking. Macromol Chem Phys. 2002; 203:1466–72.
68. Cellesi F, Tirelli N, Hubbell JA. Towards a fully-synthetic substitute of alginate: development of a new process using thermal gelation and chemical cross-linking. Biomaterials. 2004; 25:5115–24. [PubMed: 15109835]
69. Cellesi F, Tirelli N. A new process for cell microencapsulation and other biomaterial applications: Thermal gelation and chemical cross-linking in “tandem”. J Mater Sci Mater Med. 2005; 16:559–565. [PubMed: 15928872]
70. Jeong BM, Lee DS, Bae YH, Kim SW. Biodegradable block copolymers as injectable drug-delivery systems. Nature. 1997; 388:860–862. [PubMed: 9278046]
71. Jeong BM, Lee DS, Bae YH, Kim SW. *In situ* gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. J Biomed Mater Res. 2000; 50:171–177. [PubMed: 10679681]
72. Chen S, Singh J. Controlled delivery of testosterone from smart polymer solution based systems: In vitro evaluation. Int J Pharm. 2005; 295:183–190. [PubMed: 15848003]
73. Zitzmann M, Nieschlag E. Hormone substitution in male hypogonadism. Mol Cell Endocrinol. 2000; 161:73–88. [PubMed: 10773395]
74. Lee J, Bae YH, Sohn YS, Jeong BM. Thermogelling aqueous solutions of alternating multiblock copolymers of poly(L-lactic acid) and poly(ethylene) glycol. Biomacromolecules. 2006; 7:1729–1734. [PubMed: 16768391]
75. Behravesh E, Shung AK, Jo S, Mikos AG. Synthesis and characterization of triblock copolymers of methoxy poly(ethylene glycol) and poly(propylene fumarate). Biomacromolecules. 2002; 3:153–158. [PubMed: 11866568]
76. Fisher JP, Jo S, Mikos AG, Reddi AH. Thermoreversible hydrogel scaffolds for articular cartilage engineering. J Biomed Mater Res A. 2004; 71:268–274. [PubMed: 15368220]
77. Kang GD, Cheon SH, Song SC. Controlled release of doxorubicin from thermosensitive poly(organophosphazene) hydrogels. Int J Pharm. 2006; 319:29–36. [PubMed: 16677786]
78. Kang GD, Cheon SH, Khang G, Song SC. Thermosensitive poly(organophosphazene) hydrogels for a controlled drug delivery. Eur J Pharm Biopharm. 2006; 63:340–346. [PubMed: 16527468]
79. Park KH, Song SC. Morphology of spheroidal hepatocytes within injectable, biodegradable and thermosensitive poly(organophosphazene) hydrogel as cell delivery vehicle. J Biosci Bioeng. 2006; 101:238–242. [PubMed: 16716925]
80. Sohn YS, Kim JK, Song R, Jeong B. The relationship of thermosensitive properties with structure of organophosphazenes. Polymer. 2004; 45:3081–3084.



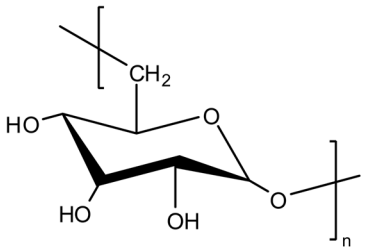
R=CH_3 or H

a) methylcellulose

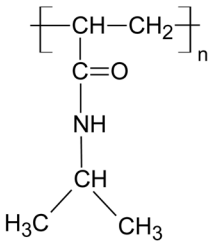


R=NH_2 or 

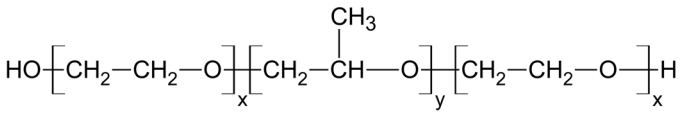
b) chitosan



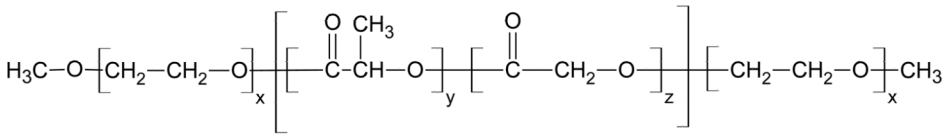
c) dextran



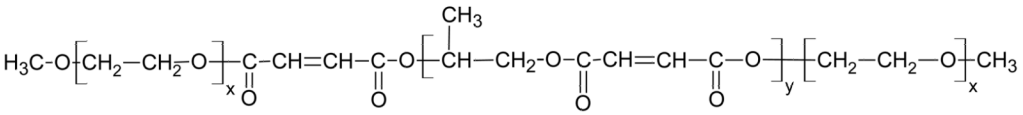
d) pNiPAAm



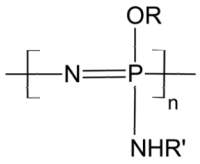
e) PEO-PPO-PEO (Pluronics®)



f) PEG-PLGA-PEG



g) PEG-PPF-PEG



R and R' can vary

h) general structure of poly(organophosphazenes)

Figure 1.
Chemical formulas of polymers that form or are part of thermoresponsive hydrogels

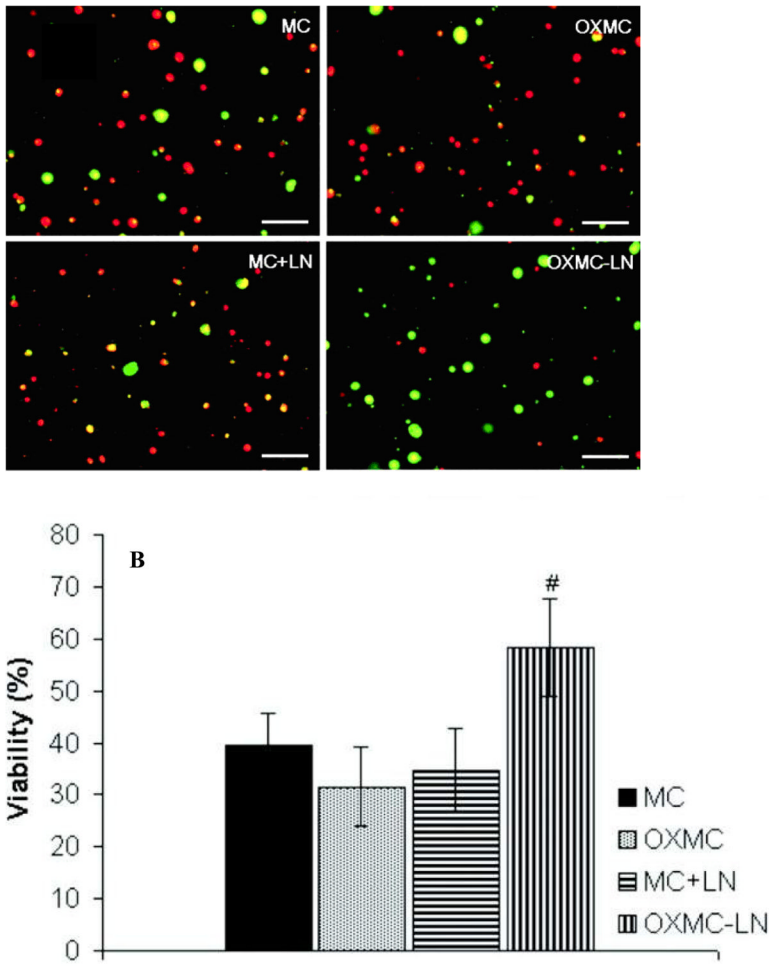


Figure 2.

Viability of neurons plated within methylcellulose scaffolds. A: Representative micrograph images from 100-μm thick confocal scans of 3-D neuronal cultures in MC, OXMC, MC + LN, and OXMC-LN at 1 day post-plating. Live cells: green, Dead cells: red. Scale bar = 50 μm. B: A significant increase in cell viability was observed with OXMC-LN when compared to MC, OXMC, and MC + LN, which did not show statistically significant differences from each other ($p < 0.01$). Reproduced from [22] with permission.

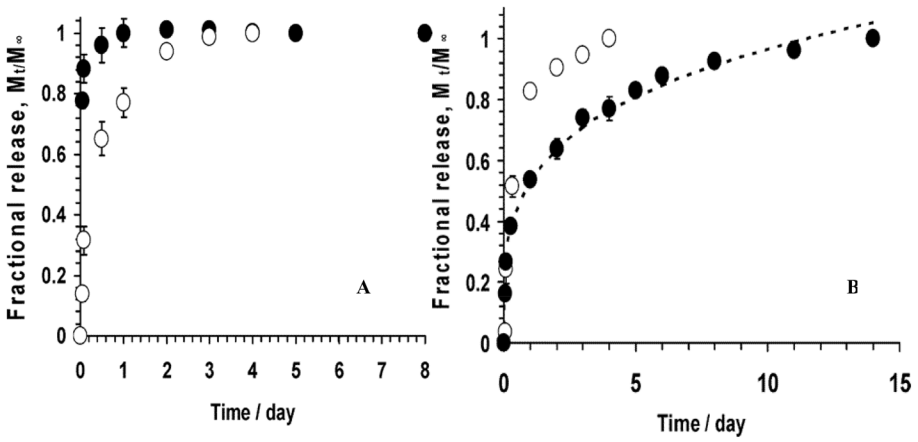


Figure 3.
Fractional release from a hydrogel with 5:4 NiPAAm:Dex-lactate-HEMA weight ratios in PBS of methylene blue (A) and BSA (B) at 25 (o) and 37(●)° C. Reproduced from [31] with permission.

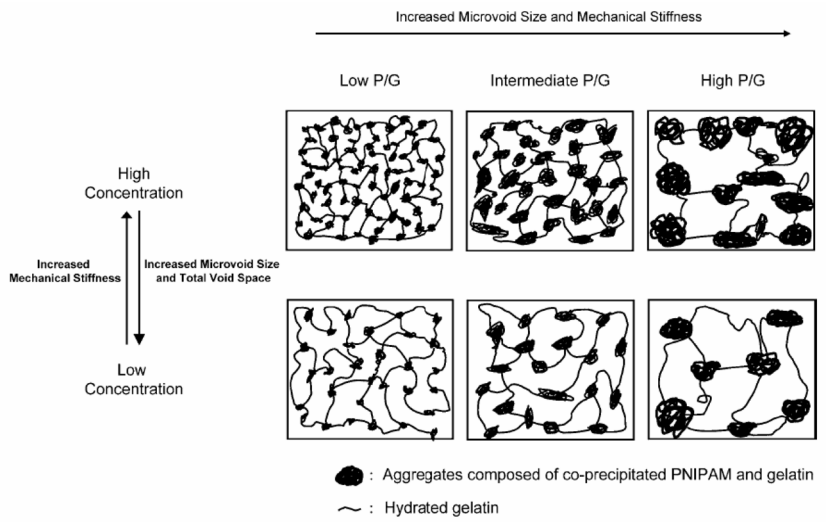


Figure 4.
Structure of pNIPAAm-gelatin (P/G) hydrogels as a function of P/G ratio and concentration. Reproduced from [35] with permission.

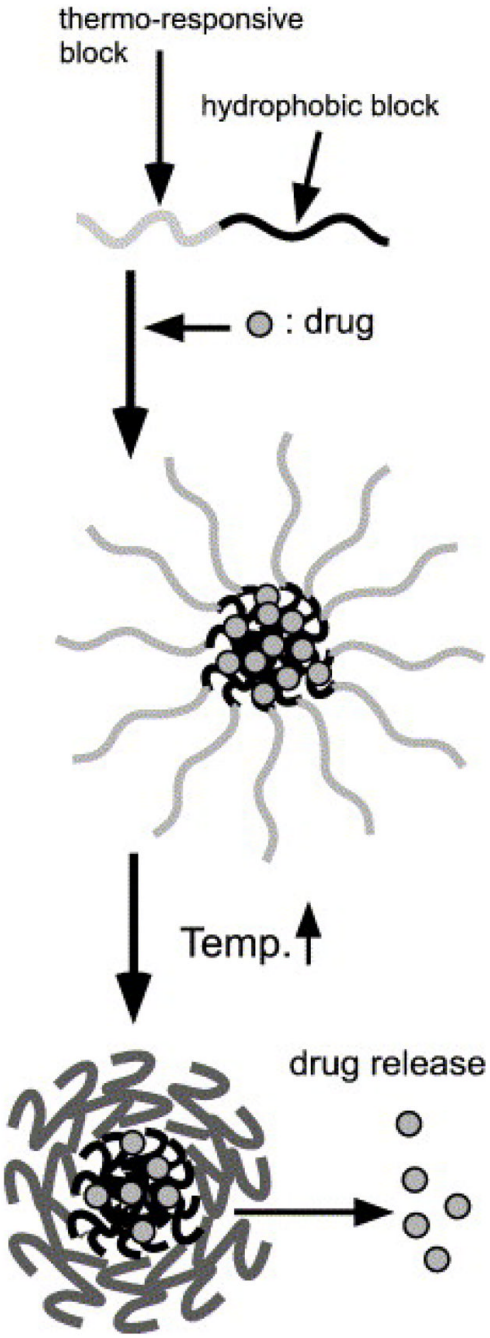


Figure 5.
Drug release from a thermosensitive polymer micelle upon temperature increase. Reproduced from [37] with permission.

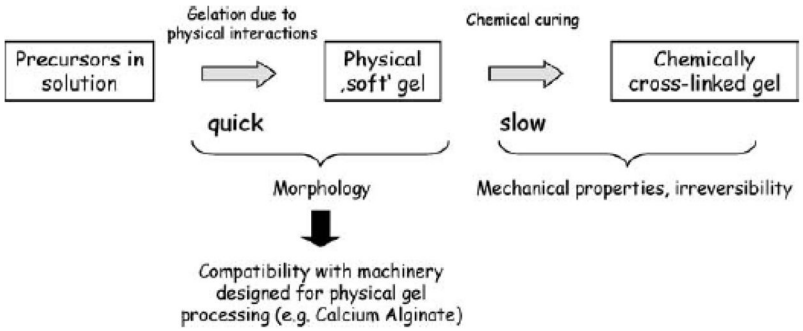


Figure 6.
Illustration of the “tandem” gelation process. Reproduced from [69] with permission.

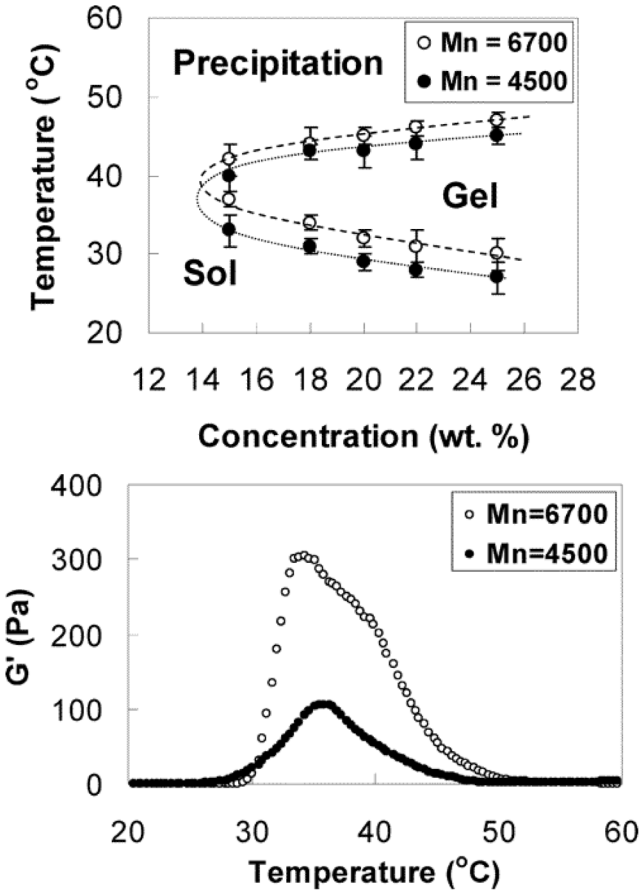


Figure 7.
Influence of total molecular weight of PEG/PLLA multiblock copolymers on the sol-gel transition temperature and storage modulus in aqueous solutions. The modulus was determined for a polymer concentration of 25 wt %. Reproduced from [74] ith permission.