ACS Biomaterials SCIENCE & ENGINEERING

Review

pubs.acs.org/journal/abseba

Thermogels: In Situ Gelling Biomaterial

Sing Shy Liow,[†] Qingqing Dou,[†] Dan Kai,[†] Anis Abdul Karim,[†] Kangyi Zhang,[†] Fujian Xu,[^,#] and Xian Jun Loh*[,†,§,⊥]

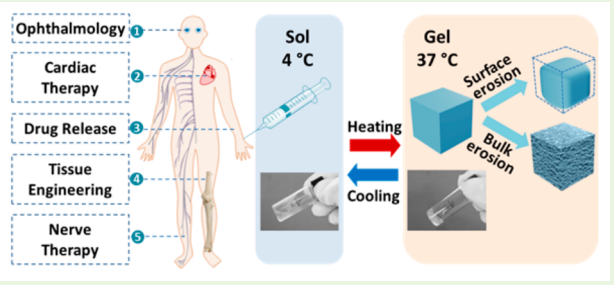
[†]Institute of Materials Research and Engineering (IMRE), 2 Fusionopolis Way, #08-03 Innovis, Singapore 138634

[^]Key Laboratory of Carbon Fiber and Functional Polymers, Ministry of Education, College of Materials Science & Engineering, [#]Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, Beijing 100029, China

[§]Department of Materials Science and Engineering, National University of Singapore, 9 Engineering Drive 1, Singapore 117576, Singapore

[⊥]Singapore Eye Research Institute, 11 Third Hospital Avenue, Singapore 168751, Singapore

ABSTRACT: In situ gel delivery systems are preferred over conventional systems due to sustained and prolonged release action of therapeutic payload onto the targeted site. Thermogel, a form of in situ gel-forming polymeric formulation, undergoes sol−gel transition after administration into the body. At room temperature, the system is an aqueous polymer solution that easily entraps therapeutic payload by mixing. Upon injection, the higher physiological temperature causes gelation in situ because of the presence of thermosensitive polymers. The gel degrades gradually over time, allowing sustained release of therapeutics localized to the site of interest. This minimizes systemic toxicity and improved efficacy of drug release to the targeted site. Thermogel properties can be easily altered for specific applications via substitution and modification of components in diblock and triblock copolymer systems. The feasibility of fine-tuning allows modifications to biodegradability, biocompatibility, biological functionalization, mechanical properties, and drug release profile. This review summarized recent development in thermogel research with a focus on synthesis and self-assembly mechanisms, gel biodegradability, and applications for drug delivery, cell encapsulation and tissue engineering. This review also assessed inadequacy of material properties as a stand-alone factor on therapeutic action efficacy in human trials, with a focus on OncoGel, an experimental thermogel that demonstrated excellent individual or synergistic drug delivery system in preclinical trials but lacked therapeutic impact in human trials. Detailed analysis from all aspects must be considered during technology development for a successful thermogel platform in drug delivery and tissue engineering.



KEYWORDS: *injectable hydrogels, thermosensitive polymers, LCST, sol−gel transition*

INTRODUCTION

Hydrogel is an important class of soft material that is suitable for a wide range of biomedical applications because of its high water content and tunable properties. These hydrophilic three-dimensional polymeric networks formed by chemical or physical cross-links can hold a large amount of water without disintegration. Chemically cross-linked hydrogels are typically tough and elastic, creating highly demanded properties in dynamic environments such as skin, cartilage, and cardio-related devices.[1] In contrast, hydrogels based on physically cross-linked polymeric networks (e.g., molecular self-assembly, hydrogen bonding, hydrophilic/hydrophobic interaction, host−guest inclusion complex),[2] are formed via simple phase transition (sol−gel) in water without any chemical reaction or external energy source. This system is particularly attractive due to simple physical phase transition and safety in in vivo experiments.[3]

Thermoresponsive hydrogel, also known as thermogel, undergoes physical sol−gel transition as temperature changes, which is reversible upon cooling. Thermogel can be easily administered via injection using conventional syringe and subsequent in situ gelation occurs at physicological temperature.[4] A typical injectable thermogel system is formulated by simple mixing of drug in hydrogel below the gel transition temperature. After injection, sol-to-gel transition occurs to transform the minimally viscous solution into a drug delivery gel depot. This method is advantageous because (i) it avoids invasive surgery for implantation; (ii) the hydrogel has a high water content that improves compatibility with the injection site; (iii) sterilization is done easily by syringe filtration; (iv) peptides are encapsulated at low temperature, which prevents denaturation due to organic solvent interaction or high temperature dissolution; (v) the biodegradable thermogel can be excreted from the body after achieving its intended purpose; (vi) the rate of drug release can be easily tailored by changing the formulation.[4c]

Received: December 3, 2015
Accepted: January 17, 2016
Published: January 18, 2016

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

The objective of this review is to summarize the different thermogels developed recently with a focus on synthesis and self-assembly mechanisms, gel biodegradability, and applications for drug delivery, cell encapsulation, and tissue engineering. Lessons learned from OncoGel development and other current cancer treatment formulations are also highlighted.

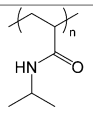
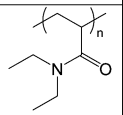
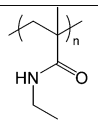
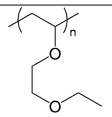
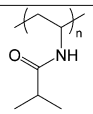
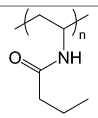
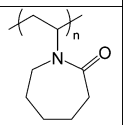
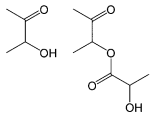
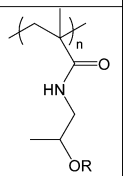
1. Synthesis and Self-assembly of In Situ Forming Gels. Thermogels exhibit reversible sol−gel transition as temperature changes. This phase change behavior is reversible as the gel is formed by physical cross-links between the polymer chains (except for some chitosan derivatives). These thermoresponsive copolymers consist of hydrophilic and hydrophobic segments, which can self-assemble into polymeric micelles in water. The hydrophobic segments form the core of the micelles while the hydrophilic chains interact with water molecules at the corona. Unlike chemical cross-links, which are irreversible, these thermoresponsive copolymers are formed via hydrophilic/hydrophobic physical associations.

Thermoresponsive copolymers can be synthesized via several methods including ring opening polymerization, atom transfer radical polymerization (ATRP), reversible addition−fragmentation chain transfer (RAFT) polymerization and polyurethane formation (polycondensation). Each method aims to produce amphiphilic copolymers that consist of hydrophilic and hydrophobic segments. As gel formation is mainly driven by hydrophobic attraction, fine-tuning the ratio of hydrophilic and hydrophobic segments is the key to achieving thermogelling property. Poly(ethylene glycol) (PEG) and poly(propylene glycol) (PPG) are commonly used in thermogels because of their well-known biocompatibility. PEG has a lower critical solution temperature (LCST) in the range of 100−150 °C in water, while PPG has a LCST range of 10−30 °C in water. In other words, they form solution below the LCST, and precipitate above this temperature. With PEG as the hydrophilic segment and PPG as the hydrophobic segment, this amphiphilic copolymer shows thermogelling behavior at physiological temperature. Besides PEG and PPG, typical polymers that exhibit LCST include poly(*N*-isopropyl acrylamide) (PNIPAAM), poly(vinyl ether) (PVE), poly(*N*,*N*-diethylacrylamide) (PDEAM), poly(*N*-vinyl alkyl amide), and poly(*N*-vinyl caprolactam), as listed in Table 1.[5] Roy and coworkers listed a comprehensive table of various thermoresponsive polymers and their respective LCST and upper critical solution temperature (UCST).[6] There are few studies describing about the hydrogels that consisted of polymers with UCST behavior. These polymer-water systems form gel below UCST and become solution above it. Ward at al. discussed phase change behavior of these polymers.[7] For example, gelatin (UCST about 30 °C), copolymers of poly(acrylamide) and poly(acrylic acid),[8] poly(N,N-dimethyl-(acrylamidopropyl) ammonium propanesulfonate),[9] copolymers of poly(allyurea) and poly(L-citrulline),[10] copolymers of poly(allyurea) and poly(allyamine),[11] poly(*N*-acryloyl glycinamide).[12]

1.1. PEG-based Block Copolymers via Ring-Opening Polymerization. PEG−PPG−PEG triblock copolymers (ABA type), also known as Pluronic (BASF) or Poloxamer (ICI) consist of 30% PPG hydrophobic segment and 70% PEG hydrophilic segment. Long-term drug release profile is not feasible as these gels erode within a few days in vivo. Numerous studies have provided various alternative modifications, including cross-linking,[13] grafting,[14] copolymerization[15] or substituting PPG to other polyesters such as PLGA,[16] PCL,[17] and poly([R]-3-hydroxybutyrate) (PHB)[18] (Figure 1, **1**−**4**). These ABA type triblock copolymers are synthesized in a two-step reaction: ring opening polymerization of B block using methoxy-PEG as initiator, followed by condensation reaction to link two B blocks together using diisocyanate as coupling agent.

Substituting PPG block with more hydrophobic segment will affect the overall physical nature of the polymer. Replacing PPG with PLGA significantly increased the sustainment of gel duration for up to a few weeks. Increasing hydrophobicity enhances thermodynamic interaction associated with gelation. As demonstrated by the increased of hydrophobic PLGA chain length, the gelation temperature and gelation concentration decreased. By substituting with PCL, which is more hydro-

Table 1. LCST of Several Typical Thermoresponsive Polymers[5]

Polymer	Chemical Structure	LCST (°C)
poly (N-isopropyl acrylamide) (PNIPAAm)		32
poly(N,N-diethyl acrylamide) (PDEAM)		25
poly(N-ethyl methacrylamide) (PNEMAM)		58
poly(methyl vinyl ether) (PMVE)		34
poly(2-ethoxyethyl vinyl ether) (PEOVE)		20
poly(N-vinyl isobutyramide) (PNVIBAM)		39
poly(N-vinyl n-butyramide) (PNVBAM)		32
poly(N-vinyl caprolactam) (PNVCa)		30-50
Poly (hydroxypropyl methacrylamide) (HPMA) derivatives e.g. PHPMA mono-lactate/dilactate when R = 		65 (mono-lactate) 13 (dilactate)

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

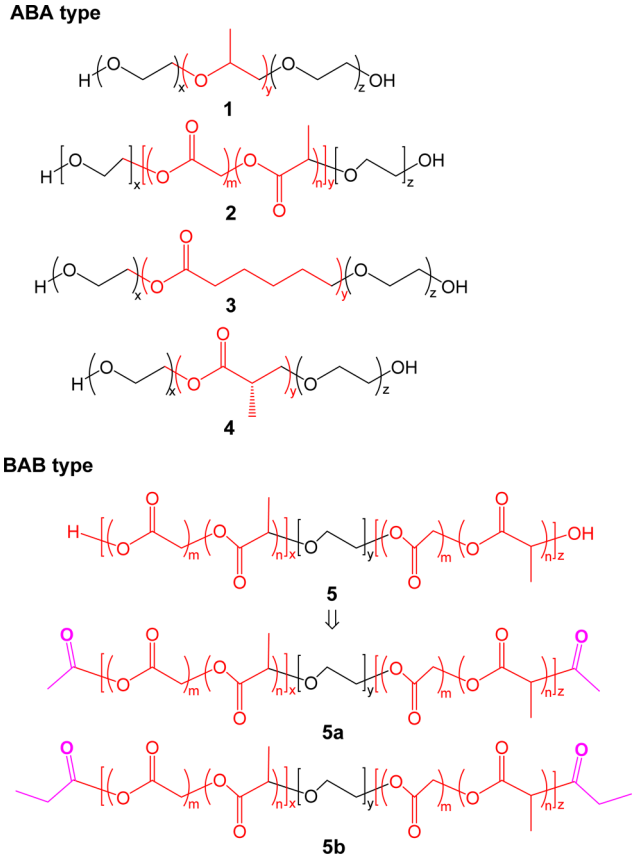


Figure 1. Chemical structure of ABA type and BAB type triblock copolymers (red, hydrophobic segments; pink, additional hydrophobic ends; blue, pH-responsive segments). **1**, Pluronic PEG−PPG−PEG; **2**, PEG−PLGA−PEG; **3**, PEG−PCL−PEG; **4**, PEG−PHB−PEG; **5**, PLGA−PEG−PLGA; **5a**, diacetate PLGA−PEG−PLGA; **5b**, dipropionates PLGA−PEG−PLGA.

phobic than PLGA, PEG−PCL-PEG forms a gel at a lower polymer concentration as compared to PEG−PLGA-PEG. PLGA (G:L ratio 2:8) and PCL exhibits three and ten times more hydrophobicity respectively than PPG.[17b] Li et al. studied PEG−PHB-PEG, in which PHB has typically higher hydrophobicity than most biodegradable polyesters.[18] Although the system can form micelles in an aqueous environment, thermogelation is not achievable at any temperature, possibly because of the imbalanced hydrophilic−hydrophobic ratio.

BAB-type triblock thermogels, especially PLGA−PEG−PLGA, have been studied intensively since 2000.[19] The synthesis of BAB type amphiphilic copolymers is easier as compared to the ABA type. PLGA−PEG−PLGA (1500−1000−1500) thermogel (commercially available as ReGel) is used in release studies of proteins and conventional drugs.[19c] It is prepared by ring opening polymerization of lactic acid and glycolic acid cyclic monomers, using PEG 1000 Da as the initiator and tin octoate as the catalyst. Sol−gel transition of PLGA−PEG−PLGA (BAB type) occurs at a lower temperature due to enhanced hydrophobic interaction which accounts from more hydrophobic segments in the system compared to PEG−PLGA−PEG (ABA type). Furthermore, modifications on the end groups from hydrophilic hydroxyl terminals to hydrophobic alkyl chains (CH_2CH_3) significantly decreases the sol−gel transition temperature by 10 °C.[19a,20] The gelation concentration also decreases from 12 to 2 wt % (Figure 1, **5a** and **5b**). The research group also reported end group effect on thermogelling properties with ionizable-end group, affecting hydrophilic/hydrophobic balance.[21] These studies draw a clear conclusion: tuning the balance of hydrophilicity and hydrophobicity is the key to achieving thermogelation.

Physical gelation of PLGA−PEG−PLGA thermogel in water was studied using TEM, ^{13}C NMR and DLS,[19a] the physical observation and schematic drawing of micelles network were shown in Figure 2. Gelling mechanism was affected by ordered-

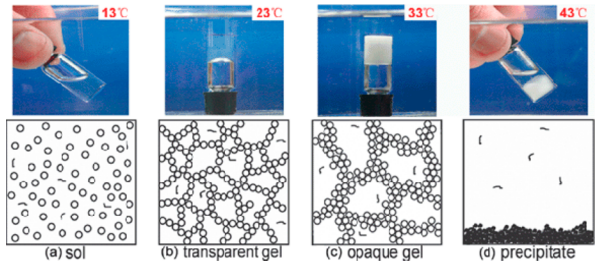


Figure 2. Visual observation (above) and schematic drawing of PLGA−PEG−PLGA micellar network showing thermogelling behavior (below). Reproduced with permission from ref 19b. Copyright 2008 Royal Society of Chemistry.

packing of micelles. At sol state, micelles were formed by self-assembly of amphiphilic block copolymers, with hydrophobic PLGA and hydrophilic PEG form the core and the corona, respectively. As temperature increases to the sol−gel transition temperature, the micelles aggregate into percolated micellar network via hydrophobic interactions.[22] The gel turns opaque as the micelle aggregates grow into a network with a mesh size of visible light wavelength. As temperature continues to rise, excessive hydrophobicity destroys the micellar structure which leads to macroscopic precipitation.

As mentioned above, thermogelling properties could be affected by hydrophobic block type, block sequence (ABA and BAB), and end groups. Reports have also shown that thermogelling properties could be adjusted by molecular weights and polydispersity indices of both hydrophilic and hydrophobic blocks.[23] Addition of salts (e.g., NaCl) can significantly tune the sol−gel transition temperature and critical gelation concentration of triblock copolymers.[16] Mixing of two nonthermogellable copolymers can sometimes lead to thermogellation.[24]

Recently, studies on PF127 conjugation revealed possible tunable properties. Shachaf et al. reported the synthesis of PF127 and fibrinogen cross-linked hydrogel, prepared by photopolymerization of acrylated PF127.[13a] Another study reported PF127 double-cross-linked network prepared via physical mixing of PF127 gels and carboxymethyl chitosan in the presence of glutaraldehyde. At physiological temperature, the glutaraldehyde carboxymethyl chitosan cross-links formed interpenetrate the PF127 gel.[13b] Moreno et al. demonstrated that mechanical and bioadhesive properties of PF127 gel significantly improved after conjugation with poly(methyl vinyl ether-*co*-maleic anhydride) (Gantrez) via ring-opening polymerization.[15]

1.2. PEG-(oligo-peptides) Block Copolymers via Ring Opening Polymerization. PEG-oligo-peptides block copolymers, also known as poly(phosphazene)s, is a class of thermosensitive polymers prepared by ring opening polymerization of N-carboxy anhydride using methoxy-PEG (mPEG) as

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

initiator. Transition temperatures between 25 and 98.5 °C could be obtained by varying the molecular weight of mPEG, molar ratio of the hydrophobic−hydrophilic segments, and oligo-peptides type. These copolymers are enzymatically biodegradable upon injection in vivo, but are stable during storage in aqueous condition. For example, PEG-poly(alanine-*co*-phenyl alanine) (PAF) showed thermogelling properties at low concentrations of 3−7 wt % in water.[25] The gelation mechanism is mainly driven by the dehydration of PEG at elevated temperature. Consequently, micelles aggregate with hydrophobic peptides to form the core. Transition temperature of this thermogel increases with increasing PEG chains, whereas lower transition temperatures could be achieved by using more hydrophobic oligo-peptides. Various applications in drug delivery[25] and wound healing[26] have been reported on such PEG-(oligo-peptides).

1.3. Multiblock PEG−PPG Polyurethanes via Polycondensation Reaction. Polyurethane formation is one of the easier ways to prepare thermogelling copolymers. One-pot synthesis could be carried out in the presence of polymer diols of low molecular weight and diisocyanate as the coupling agent. In a PEG/PPG polyurethane multiblock copolymer system, both PEG and PPG segments are hydrophilic below 10 °C. However, the PPG segment becomes hydrophobic above 30 °C owing to its LCST of 10−30 °C in water. It was also mentioned that LCST of PPG decreases as the molecular weight of the polymer increases.[27] Sol−gel transition of a polyurethane aqueous solution is achieved by self-assembly of hydrophilic and hydrophobic segments into micellar structure at elevated temperature.

Incorporation of a small amount (1−5%) of third component (a hydrophobic diol) into the multiblock PEG/PPG polyurethane could tune the properties of thermogelling copolymers. A study has shown that PHB added could lower the critical gelation concentration (CGC) of the aqueous system. Poly(ε-caprolactone) (PCL),[28] poly(trimethylene carbonate) (PTMC)[29] or PLA[30] imparts biodegradability, while poly(ethylene butylene)[31] provides biostability. The sol−gel transition mechanism of poly(PEG/PPG/PHB urethane)s was reported in,[32] as shown in Figure 3. The long polymer chains of PEG, PPG, and PHB are connected by urethane linkages. Associated micelles are formed by the amphiphilic multiblock copolymers at a highly diluted solution (99.9% water, 0.1% polymer). These self-assembled micelles have PEG

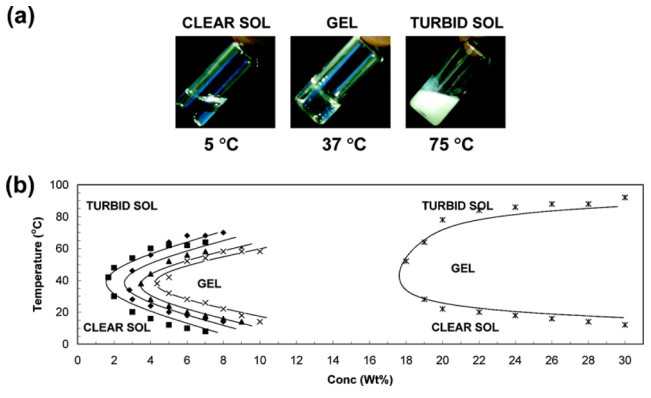


Figure 3. (a) Sol−gel transition of poly(PEG/PPG/PHB urethane)s (b) Phase-diagram of the multiblock polyurethane as compared to PF127. Reproduced with permission fro ref 33. Copyright 2007 American Chemical Society.

hydrophilic tails that interact with water and hydrophobic cores that consist of PPG and PHB. A minimum polymer concentration (2−5 wt %) and optimum micelle concentration, is necessary for gel formation. At low temperature, the aqueous solution is clear because the PPG segments behave more hydrophilic causing the polymers to be well-solvated in water. Increase in temperature resulted in the dehydration of PEG segments, and PPG segments behave more hydrophobic above its LCST, becoming less water-soluble. When hydrophobic/hydrophilic balance in the system is achieved, a gel state is reached. Micellar aggregation due to self-association of PEG corona and increased hydrophobicity of PPG drives the gel formation. Increase in temperature resulted in severe dehydration and collapse of the PEG corona, exposing the hydrophobic core to form a turbid solution. PHB added as a third diol component in this system enhanced the hydrophobicity, leading to a lower CGC as compared to poly(PEG/PPG urethane).

1.4. Poly(N-isopropyl acrylamide)-Based Block Copolymers via ATRP and RAFT Polymerization. Poly(*N*-isopropyl acrylamide) (PNIPAAm) pioneered the development of reversible thermosensitive hydrogel. Since 1960s, numerous studies on synthesis of thermoresponsive PNIPAAm and its derivatives have been reported for biomedical applications such as drug delivery, cell encapsulation and cell culture sheets.[34] PNIPAAm known for having low LCST at 32 °C in aqueous solution is relatively insensitive to the changes in pH, concentration or chemical environment.[35] Below its LCST, the polymer is hydrophilic and water-soluble; at LCST, it exhibits reversible phase change to a hydrophobic state causing the polymer structure to collapse from coil to globule and precipitates out of the aqueous solution.

ATRP technique is the most effective and widely used method to polymerize (meth)acrylates, (meth)acrylamides, styrene and their copolymers.[36] Hence, ATRP is ideal for the synthesis of most PNIPAAm-based hydrogels. Complex polymer structure (especially graft copolymers) with narrow polydispersity index can be obtained. The polymerization mechanism involves dynamic equilibrium between the active species activated by redox active transition metal complexes and dormant species. Thermoresponsive PNIPAAm-[hydrophobic core]-PNIPAAm and PNIPAAm-[hydrophilic core]-PNIPAAm copolymers can be obtained by ATRP.[37] PNIPAAm copolymerized with hydrophobic segments leads to a lower LCST than PNIPAAm homopolymers. These copolymers are not suitable for in situ gelling applications but can act as nanocarriers which release hydrophobic content at the targeted site as the copolymers precipitate or shrink. In contrast, copolymerization of PNIPAAm with hydrophilic segments increases the overall hydrophilicity, thus increasing the LCST. These copolymers are suitable for injectable in situ gelling applications.

Lignin-*g*-PNIPAAm copolymers (lignin as the hydrophobic core) precipitates and forms a gel above PNIPAAm LCST of 32 °C.[37b] In contrast, PNIPAAm−PMPC-PNIPAAm triblock copolymers with hydrophilic poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) segments forms a gel at 37 °C. This gelation mechanism relies on hydrophobic interactions between PNIPAAm blocks at temperatures above LCST.[37d] Hence, the triblock, instead of diblock conformation is essential for the occurrence of intermicellar bridging. Recently, Li et al. prepared lignin-*b*-PNIPAAm-*b*-(PEG-*co*-PPG) copolymers consisting of lignin as the hydrophobic core, PEG as the

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

hydrophilic corona, and PPG and PNIPAAm as thermosensitive segments. These thermosensitive segments transform from hydrophilic to hydrophobic as the temperature increases.[38] The thermogel prepared by ATRP showed sol−gel transition at 33−35 °C and precipitation at 52 °C. Interestingly, the thermogel has a low critical gelation concentration (CGC) at 1.3 wt % of polymer in 98.7 wt % of water. Low polymer concentration is advantageous for in situ gelling compatibility and cost effectiveness.

RAFT polymerization is another method employed to obtain multiblock amphiphilic copolymers.[39] As compared to ATRP multistep alternating addition of two types of monomers into a living polymerization system, RAFT polymerization is relatively easier to prepare with narrow polydispersity index. Using cyclic- or polytrithiocarbonates as the chain transfer agent, a multiblock PNIPAAm-PDMA copolymer was prepared with a two-step addition, as shown in Figure 4. The chain length of

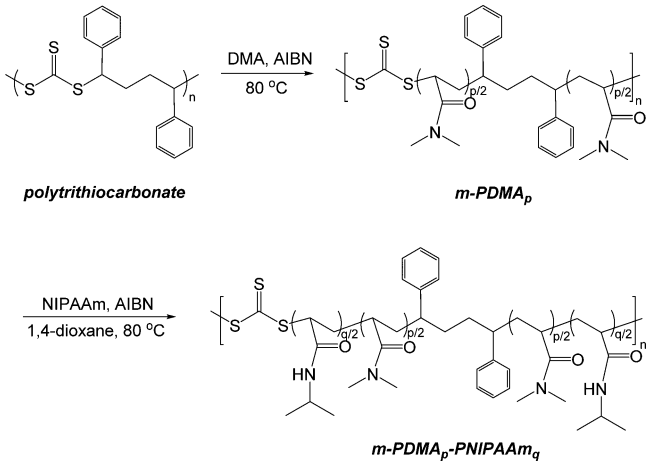


Figure 4. Synthesis of multiblock copolymers m−PDMA-PNIPAAm by successive RAFT polymerization, using polytrithiocarbonate as chain transfer agent. Reproduced with permission from ref 42. Copyright 2011 American Chemical Society.

each sequence can be tuned by the ratio of monomer and trithiocarbonates.[40] Recent study highlighted the feasibility to prepare low LCST blocks of PNIPAAm and poly(*N*,*N*-diethylacrylamide) (PDEA) in an aqueous environment at 25 °C.[41] RAFT polymerization requires no metal−ligand complex as catalyst for the polymerization; complex purification procedure can thus be avoided. Double hydrophilic block copolymers (DHBC) have been synthesized using PNIPAAm and poly(N,N-dimethylacrylamide) (PDMA) via consecutive RAFT polymerization technique.[40,42] The copolymers showed thermally induced unimolecular or multimolecular micelles aggregation based on different copolymer architecture, as shown in Figure 5. Physical gels are formed when the multiblock PNIPAAm-PDMA consisted of PNIPAAm and PDMA with certain sequence length, due to the formation and aggregation of unimolecular micelles. In contrast, no gel formation is observed when multimolecular micelles aggregate. Addition of salts can significantly reduce critical gelation concentration and critical gelling temperature, by establishing a correlation among the Hofmeister effect, aggregation behavior and gelation properties.[42]

1.5. Poly(oligo(ethylene glycol) methyl ether methacrylate) (PoEGMA) and Poly(oligo(ethylene glycol) acrylate via ATRP, NMP, and RAFT Polymerization. PoEGMA is a

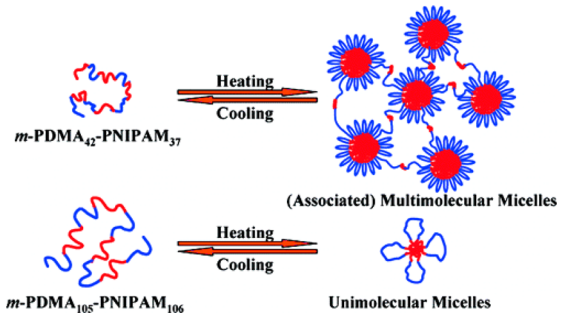
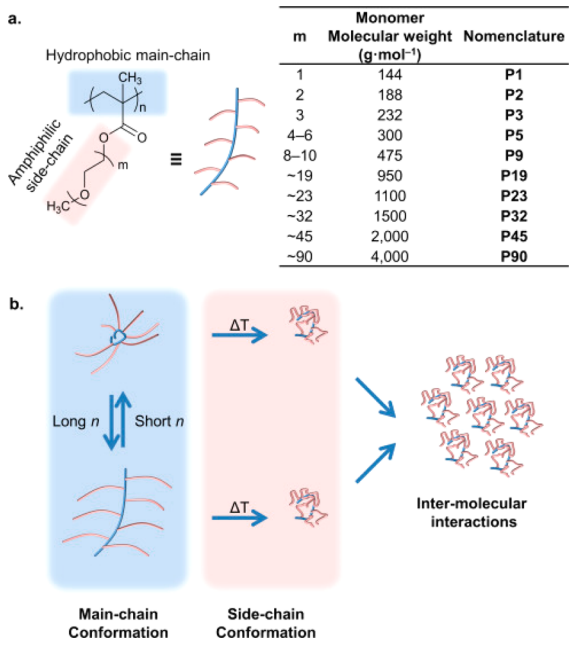


Figure 5. Schematic drawing of multiblock PDMA−PNIPAAm showing thermally induced unimolecular or multimolecular micelles aggregation (red = PNIPAAm, blue = PDMA). Reproduced with permission from ref 40. Copyright 2007 American Chemical Society.

relatively new thermoresponsive molecule discovered in the early 2000s. Lutz et al. suggested that PoEGMA-based copolymers outperform PNIPAAm because of its easily tunable LCST and biocompatibility that is comparable to linear PEG.[43] In addition to its thermosensitivity, PoEGMA shows protein-repellant properties which are of great interest as nonfouling surface applications.[44] Influence of molecular structure on the thermoresponsive properties of PoEGMA and PoEGA as biomaterials have been discussed.[45] As shown in Figure 6,



m	Monomer Molecular weight (g·mol⁻¹)	Nomenclature
1	144	P1
2	188	P2
3	232	P3
4–6	300	P5
8–10	475	P9
~19	950	P19
~23	1100	P23
~32	1500	P32
~45	2,000	P45
~90	4,000	P90

Figure 6. (a) Chemical structure of PoEGMA (main and side chains) with different number of repeating units, nomenclature, and their molecular weights. (b) Chain length effect: main chain and side chain conformation, and molecular self-assembly at elevated temperature. Reproduced with permission from ref 45a. Copyright 2015 Elsevier.

PoEGMA is a comb-shaped polymer with a hydrophobic backbone and hydrophilic side chains. The LCST is tunable via the relative chain length of the main and side chains, as well as the end group functionalities. Well-defined molecular architecture such as polymer brush and amphiphilic block copolymers can be synthesized by controlled radical polymerization including ATRP, NMP (nitroxide-mediated radical polymerization) and RAFT techniques.[46] PoEGMA showed a

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

range of LCST (P2 to P9, in Figure 5) from 26 to 90 °C depending on the number of PEG side chain repeating units.[47]

2. Evaluating the Biodegradability of Thermogels. Biodegradability of thermosensitive hydrogels has received much research attention for its degradation property after serving its effective purpose as therapeutic delivery vehicle in the body. Introducing biodegradable linkages into the polymer backbone facilitates degradation of the copolymers into smaller fragments and subsequent excretion from the body. Significant number of publications in controlled drug delivery has shown the importance of evaluating biodegradability of thermogels in vitro and in vivo. Biodegradability plays an important role on efficiency of drug delivery because the drug release profile is affected by gel erosion, degradation and diffusion mechanisms. To understand the biodegradation of thermogels, characterization techniques such as SEM, GPC, ^{1}H NMR, MALDI-TOF, and TGA are typically used to provide visual analysis, molecular measurement, mass loss and structural integrity of the thermogels after incubation in vitro and in vivo.

2.1. Biodegradable Thermogels. PEG−PPG-PEG triblock copolymer (or Pluronics), an FDA approved drug delivery system, is the most common thermogelling copolymer studied.[48] However, limitation of these polyethers-based thermogelling copolymers lies on the nonbiodegradable carbon−carbon backbone that could lead to bioaccumulation in the body. Furthermore, the gel retention period is shortened by gel erosion that occurs in vivo within a few days resulting in unfavorable sustained drug delivery. To impart biodegradability, hydrolytic degradable polyesters such as poly(L-lactic acid) (PLLA), poly(ε-caprolactone) (PCL), poly([R]-3-hydroxybutyrate) (PHB), poly(D,L-lactide-*co*-glycolide) (PLGA) were being employed as degradable blocks.[4c,16,17b,49] In addition, polypeptide-based thermogelling systems containing enzymatically degradable peptides such as poly(alanine-*co*-phenyl alanine) (PAF) and poly(L-alanine) (PAL) were used in the synthesis of thermoresponsive copolymers. These systems demonstrated good in vitro stability in aqueous solution and in vivo degradability at the presence of proteolytic enzymes.[50]

2.2. Rate of Degradation. The core motivation behind controlled drug delivery is achieving tunable rate of degradation in vitro and in vivo. Rate of degradation can be tailored by (1) block length, (2) end group modification, (3) type of biodegradable block, (4) composition of the copolymers, (5) environmental effect. Tailoring these components could affect drug release rate and drug delivery profile. Some studies revealed that different physiological environment (presence of enzymes, ionic exchange and constant flow of body fluid) in vitro and in vivo could affect degradation profiles. This subsection will discuss research on degradation of thermogels in both in vitro and in vivo.

In vitro degradation rate of thermogelling PEG−PLGA−PEG copolymers is affected by the block length of hydrophobic component (2310 and 2810 Da) and polymer concentration in water (20, 27, and 33 wt %). Thermogels with longer hydrophobic block (i.e., PLGA) or higher polymer concentration in water, resulted in slower degradation.[51] In vivo degradation demonstrated rapid sol−gel transition (33 wt % of polymer in aqueous solution) upon subcutaneous injection in the rat with stable three-dimensional gel shape maintained for more than 1 month.[52]

Kim et al. reported on sulfamethazine oligomers (SMOs) added to both ends of thermosensitive PCLA−PEG−PCLA block copolymer to impart pH- and thermo-sensitivity.[53] In vitro evaluation on block copolymer-SMO gel showed slower degradation rate due to buffering effect of sulfonamide groups. The presence of sulfonamide groups neutralized the accelerated degradation effect as a result of accumulation of degradation products e.g. lactic acid and caproic acid from PCLA segment. In vivo degradation of the block copolymer−SMO gel showed complete degradation within 6 weeks.

In vitro degradation studies of thermogelling poly(ester urethane)s based on PEG and PPG were reported. PHB, PLLA, or PCL are added on these PEG/PPG-based poly(ester urethane)s to impart biodegradability.[28,49b,54] Different rates of degradation can be achieved by changing the degradable polyester groups and composition in the thermogelling copolymers. Copolymer gel (5 wt % polymer in aqueous solution) consisted of PHB segment could be completely eroded in 30, 40, and 70 days, inversely proportional with PHB content of 8, 5, and 2 wt %, respectively. Structural deterioration was observed at day 14, as visualized by SEM analysis (Figure 7). However, the molecular weight of the copolymer (M_n) remained half of the initial M_n (~60 000 Da) after six months of incubation.[54] These observations suggested that the copolymer gel consisted of PHB follows a typical surface degradation model. Typical surface degradation of polyesters proceeds with mass loss at constant velocity, with molecular weight decrement observed at the later stage,[55] which is similar to the poly(PEG/PPG/PHB) thermogel. Another poly(ester urethane) copolymer containing PLLA segment was synthesized using the same method.[30] The

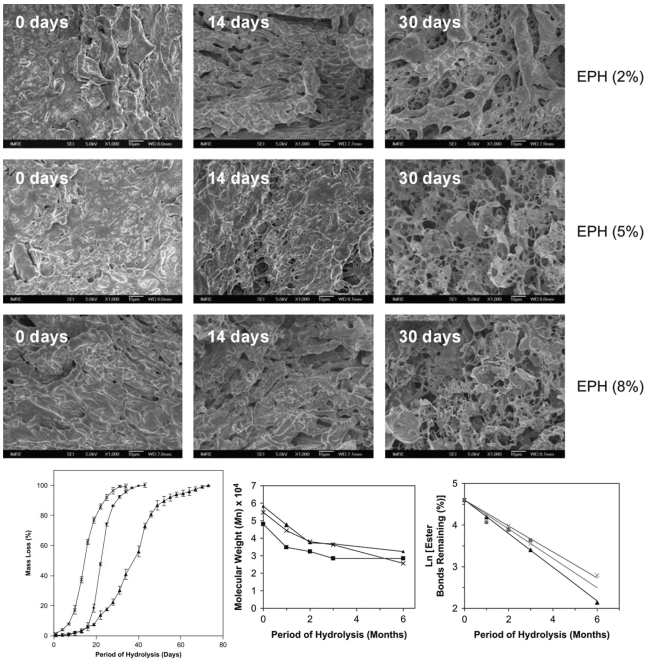


Figure 7. SEM images of hydrogel residues after various periods of degradation in PBS at pH 7.4 and 37 °C. Bottom: (left) Mass loss (%) of the poly(PEG/PPG/PHB urethane) hydrogels (5 wt %) after incubation in PBS at pH 7.4 and 37 °C. (middle) Changes in molecular weight of the copolymer degradation products with time of hydrolysis up to 6 months. (right) Plot of the natural logarithm of the fractional ester bonds remaining versus degradation time of the polymers after various periods of degradation (▲, EPH(2%); ■, EPH(5%); ×, EPH(8%)). Reproduced with permission from ref 57. Copyright 2007 Elsevier.

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

copolymer gel degraded at a much faster rate than its PHB counterpart. The gel (10 wt % polymer in aqueous solution) hydrolytically degraded to polymer fragments with M_n lower than 10 000 Da (initial M_n 25 800 Da) within three months (Figure 8). These fragments can potentially be excreted from the body via renal filtration.

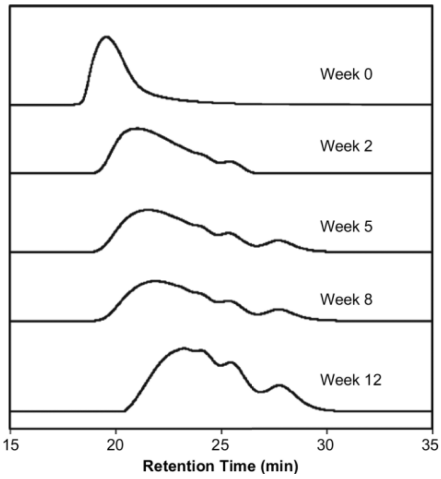


Figure 8. GPC profile of remaining poly(PEG/PPG/PLA urethane)s gels incubated in a porous cellulose cassette at various degradation intervals at pH 7.4. Reproduced with permission from ref 49b. Copyright 2008 Elsevier.

Different types of biodegradable hydrophobic blocks were studied. Thermosensitive diblock mPEG−copolyesters were prepared by ring-opening copolymerization of D,L-lactide with glycolide (from PLGA), β-propiolactone (from PPLA), δ-valerolactone (from PVLA) and ε-caprolactone (from PCLA), respectively, using methoxy-poly(ethylene glycol) (mPEG) as the initiator.[56] In vitro degradation of mPEG−copolyester gels based on weight loss within 30 days at 37 °C showed that the hydrolysis rate of hydrophobic segments primarily determined the rate of degradation (Figure 9). The study summarized the rate of degradation as follows: mPEG-*b*-PLGA > mPEG-*b*-PCLA > mPEG-*b*-PVLA > mPEG-*b*-PPLA.

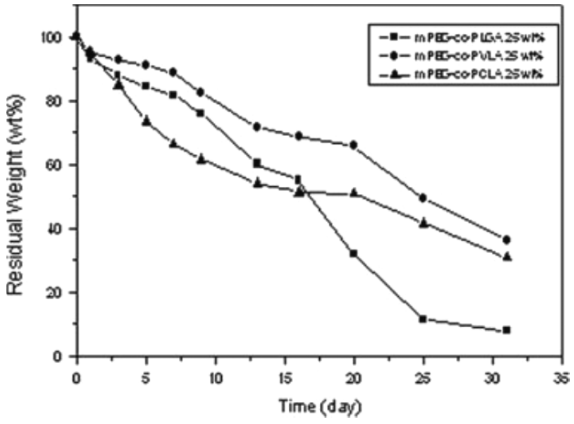


Figure 9. Degradation behavior of mPEG−polyester diblock copolymers determined using the weight loss method (●, mPEG-*b*-PVLA; ▲, mPEG-*b*-PCLA; ■, mPEG-*b*-PLGA). Degradation curve of mPEG-*b*-PPLA is not shown in figure. Reproduced with permission from ref 56. Copyright 2010 Wiley.

Studies have shown that environmental effects (e.g., concentration of glutathione, pH change) can contribute to the rate of thermogel degradation. Addition of disulfide group to Pluronics provides a glutathione concentration-sensitive erosion pattern to the thermogel.[58] No significant erosion of the thermogel in phosphate buffer saline (PBS) occurs in vitro, but the presence of high concentration of glutathione around the tumor tissue could degrade disulfide bonds in vivo. In another study, rate of degradation was tuned by adding hydroxyapatite (HA) into the thermogels.[59] In vitro study revealed that the addition of alkaline HA (60 wt %) prolonged the mass loss period and increased the pH by neutralizing the solution surrounding the hydrogel, as shown in Figure 10 a.

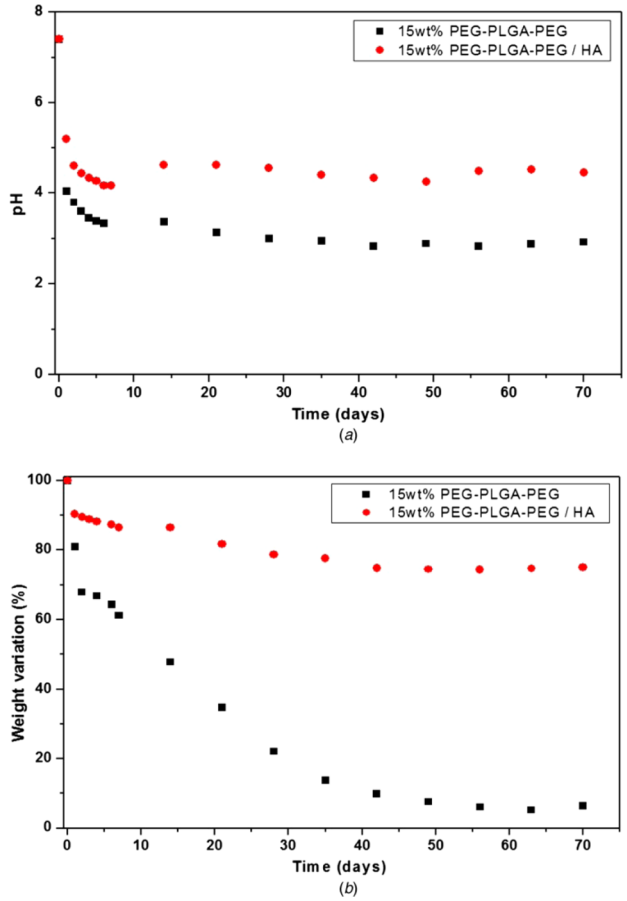


Figure 10. In vitro degradation of PEG−PLGA−PEG hydrogel and PEG−PLGA-PEG/HA hydrogel composite: (a) pH variation and (b) weight variation during incubation in phosphate buffer (pH 7.0) at 37 °C. Reproduced with permission from ref59. Copyright 2014 IOP Science.

The mass loss of the PEG−PLGA−-PEG/HA thermogel composite was claimed to be slower than the PEG−PLGA-PEG thermogel alone. In vivo study provided some insights on compatibility and tissue regeneration in relation to degradation of the thermogel. Inflammatory response appeared at 4 weeks postimplantation, attributed to the acidic degradation products originated from PLGA segments. After 8 weeks, the absence of inflammatory cells, increased presence of fibroblasts, and creation of new blood vessels suggested biocompatibility of the thermogel composite.

Studies have been reported on a group of polypeptide-based thermogels that are stable in water, but degradable in vivo by

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

proteolytic enzymes in the body. The rate of degradation is dependent on the type of polypeptides. For example, poly(ethylene glycol)-*b*-poly(alanine-*co*-phenyl alanine) (PEG-*b*-PAF)-based thermogels is stable in phosphate buffer saline but degraded in subcutaneous layer of rats.[25] The gel lost more than 90% of its original mass in 15 days of in vivo incubation, whereas negligible mass loss was observed in vitro. In a similar study, complete degradation was observed for poly(alanine-*co*-leucine)-*b*-poloxamer-*b*-poly(alanine-*co*-leucine) (PAL−PLX-PAL) gels after 47 days of in vivo incubation.[60] PEG-*b*-PAF, which degrades faster than PAL−PLX−PAL showed marginal tissue inflammation, whereas the latter showed relatively thick capsule formation around the gel.

2.3. Degradation Mechanism of Thermoresponsive Copolymers vs Thermogels. Based on discussions in the previous subsection, a variety of biodegradable thermosensitive copolymers have been developed in recent decades and their degradation profiles were carefully investigated. Several conclusions on degradation mechanism were drawn, which will be discussed in this section.

Modified PEG−PPG-PEG copolymers consisted of (1) hydrolytic degradable polyesters such as PCL, PLLA, PHB, PLGA, and (2) enzymatically degradable peptides, are two major biodegradable groups of thermogels. Hydrolysis of polyesters involves two major mechanisms: surface or bulk erosion models. Surface erosion of polyesters proceeds at constant velocity;[55] bulk erosion of polyesters changes the rate of erosion with time,[61] as depicted in Figure 11. Most

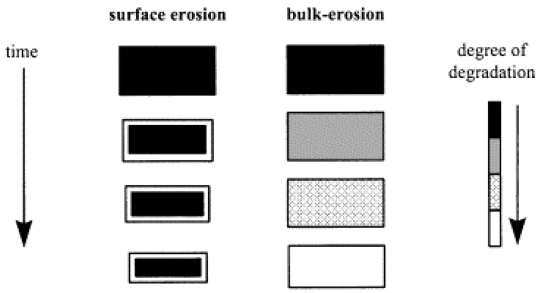


Figure 11. Schematic illustration of the changes a polymer matrix undergoes during surface erosion and bulk erosion. Reproduced with permission from ref 62b. Copyright 2002 Elsevier.

degradable polymers undergo both surface and bulk erosion but the nature and degree of degradation is dependent on three factors: (1) diffusivity of water inside the matrix, (2) degradation rate of the polymer functional groups, and (3) the matrix dimensions.[62] For example, PHB degrades via surface erosion because of its inherent high hydrophobicity that limits the diffusivity of water in the matrix, whereas PLA, PLGA, and PCL mainly exhibit bulk erosion mechanism.

Studies also revealed that degradation mechanism of thermoresponsive hydrogel is different from its original copolymer. Because of high water content, in most cases, erosion, rather than degradation, starts at the early stage of the overall incubation period. Chemically cross-linked thermogels undergo chain scission of the cross-links before polymer erosion occurs;[63] while physically cross-linked thermogels could be eroded with or without polymer degradation. In vitro hydrolytic degradation of poly(PEG/PPG/PHB)[54] was suggested to follow 3 stages (with mass loss in an "S" shape curve vs time, in Figure 7): (1) incubation period and slow dissolution at the gel surface; (2) constant mass loss due to dissociation of physical cross-links and fast dissolution of hydrogel; (3) slow mass loss toward the end of the erosion.

Physiological environment of the biological entity also affected the nature of degradation mechanism. Polypeptide-based thermogels are nondegradable in vitro, but undergo enzymatic degradation in vivo. The thermogels were incubated in PBS to study the degradation effect of proteolytic enzymes in vivo. Studies showed that PAL−PLX-PAL gels were degraded by proteolytic enzymes such as MMP-12 and elastase,[60] whereas PEG-*b*-PAF-based thermogels showed degradation at the presence of cathepsin C, cathepsin B, elastase, chymotrypsin, and collagenase.[25]

This section demonstrated various degradation studies on thermogels and their implications on physiological environment. Important take away messages include: (1) degradation of thermogels largely depends on the types of degradable block, which may results in different chemical entities postdegradation, (2) the property of degradation products may affect the environment after being released from the gel matrix, (3) degradation debris might undergo phagocytosis or pinocytosis. Therefore, that extravasation process should be carefully monitored.

3. Applications in Drug Release. Thermogelling copolymers undergo sol−gel transitions as temperatures changes. Thus, although such thermogels are injectable solutions with low viscosities at lower temperatures, they have the ability to turn into gels upon injection into the physiological environment. Biodegradable thermogels show extra benefits in their use as drug depots and delivery systems as they require no follow-up surgical removal after depletion.

3.1. Drug Loading. Hydrophobic drugs can be incorporated using the solvent-induced phase inversion technique (SPI). A water insoluble polymer is first dissolved in an organic, water-miscible solvent containing the drug. Upon its injection into the body and exposure to an aqueous environment, the organic solvent dissipates out, whereas water ingresses via diffusion.[64] This solvent exchange results in sol-to-gel transformation and polymer precipitation, leading to implant formation (Figure 12).[65]

3.2. Drug Release Rate and Mechanisms. Drug release from thermogels can be affected by several parameters, including degradability of thermogel, concentration of thermoresponsive copolymers in solution, size, hydrophobicity, pore size, concentration of a drug, and the presence of specific interactions between drug and thermogel. Generally, the release of proteins and hydrophobic drugs from a degradable hydrogel shows a two-step drug release mechanism: 1) diffusion-controlled, and 2) combination of diffusion and degradation.[54,66] On the other hand, the release of hydrophilic drugs occurs in only one step (step one).[66]

Pluronics gels are nondegradable. Furthermore, as these gels erode within a few days in vivo, long-term drug release is not feasible. As a result, a pluronic analog-based thermosetting gel for ophthalmic drug delivery was developed.[67] Pluronic analogs were incorporated with mucoadhesive polysaccharide, sodium hyaluronate (HA-Na) for ocular retention. The inclusion of F68 (10%) to F127 (21%) increased the phase transition temperature by 9 °C. The formulation was a free-flowing liquid below 25 °C and converted to a firm gel under physiological conditions. Gamma scintigraphic data demonstrated that the precorneal clearance of the thermosetting gel was significantly

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

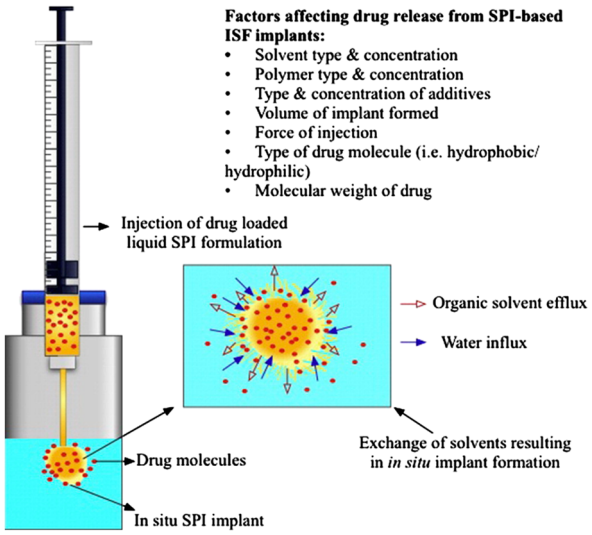


Figure 12. Schematic representations of solvent induced phase inversion technique (SPI) implant formation, solvent exchange, and drug delivery. Reproduced with permission from ref 65. Copyright 2014 Elsevier.

delayed as compared to the control solution. This means that drug release can be prolonged through the use of these gels.

The sustained release profile of protein from poly(PEG/PPG/PHB urethane) thermogels can be controlled by tuning the concentration of thermogelling copolymers in the solution, where a higher concentration of the copolymer leads to extended drug release period.[54] A PEG−PLGA−PEG (550−2310−550 and 550−2810−550) amphiphilic copolymer for drug release were reported.[51] Figure 13a showed the release profile of a hydrophobic drug (spironolactone) from the reported copolymer. Similar to poly(PEG/PPG/PHB urethane) thermogels, it was observed that a higher copolymer concentration leads to a slower drug release rate. This is because the higher initial polymer concentration resulted in a tighter close-packed structure of the gel and thus reduced pore size and permeability of the drug. However, highly concentrated polymer solutions should be avoided for drug delivery because of changes in osmolality, transparency, and kinetics of gelation of solution. Apart from initial polymer concentrations, the release rates can also be controlled by increasing the length of the hydrophobic blocks, causing a slower release rate at the degradation dominant stage due to an increased hydrophobicity in the gel. The release profile of a hydrophilic drug (ketoprofen) from the aforementioned reported copolymer was also studied by the same research group, leading to the conclusion that the release rate of hydrophilic drugs was diffusion-controlled.

The presence of specific interactions between drugs and thermogels could affect the efficacy of drug release. For example, drugs from the camptothecin family suffer severely from the problem of hydrolysis, which causes them to change from an effective antitumor form (lactone form) to an ineffective carboxylate form. Thus, the hydrolysis of drugs significantly decreases their therapeutic efficiencies and leads to severe side effects. Ding's group found the problem can be solved by mixing the drug with thermogelling triblock copolymer PLGA−PEG−PLGA[68] or PEG−PPG−PEG[69] for the delivery of camptothecin family drugs. Excellent drug efficacy was observed from these studies. For example, the sustained release of PEGylated camptothecin from the entrapped hydrogel lasted for 1 month. The efficacy of the antitumor drug was also confirmed by in vivo antitumor tests in mice.[68c] The sustained release was diffusion-controlled at the first stage and then controlled by combination of diffusion and degradation at the late stage. Later, they studied release of topotecan (TPT, a derivative of camptothecin) from encapsulated PLGA−PEG−PLGA hydrogels implants in S180-bearing mice. The in vitro release of TPT from hydrogels could be sustained for 5 days with only a mild initial burst. They also found PLGA−PEG−PLGA could enhance the activation of the inactive drug from 10% in PBS control to above 50% in the hydrogel matrix.[68b] PLGA−PEG−PLGA copolymer aqueous solution was used to deliver moderately soluble antitumor drug irinotecan (IRN).[68a] Tumor regression was observed in PLGA−PEG−PLGA and IRN treated nude mice with xenografted SW620 human colon tumors. Ding et al. also studied synthesized hydrophilic−lipophilic balance (HLB) values to the equilibrium lactone fraction ($f_{lactone}$) of the drugs with four PEG−PPG−PEG copolymers (Figure 14). The enhancement extent was significantly increased with the decrease of the copolymer HLB for weak water-soluble camptothecin drug 10-hydrocamptothecin. The effect was less significant for a more hydrophilic drug topotecan. In all cases, $f_{lactone}$ was enhanced.

In the above cases, the equilibrium fraction of active drug form (lactone) was significantly enhanced in hydrogels and even in the corresponding micelles.[69b] Apart from antitumor drugs, bovine serum albumin and glucoregulatory polypeptide-exenatide have also been delivered with PLGA−PEG−PLGA.[70] Moreover, some water-soluble drugs, such as DOX,[71] can also

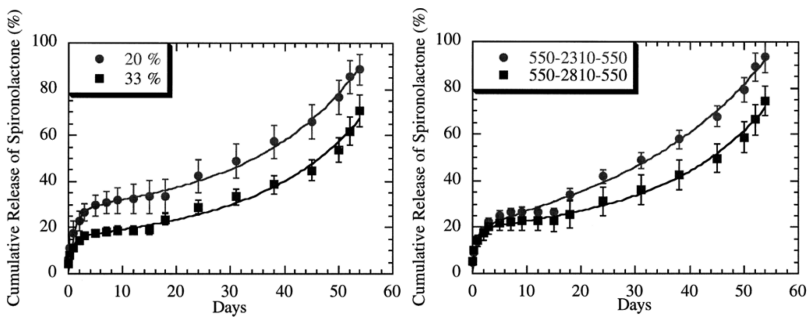


Figure 13. Release of hydrophobic drug (spironolactone) reflects the degradation rate of the polymers. Left: Higher gel hydration leads to a faster degradation rate of PEG−PLGA−PEG triblock copolymer. Right: Longer hydrophobic blocks in PEG−PLGA−PEG triblock polymers leads to smaller gel water content and slower drug release rates. Reproduced from Jeong et al.[51] Copyright 2000 Elsevier.

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

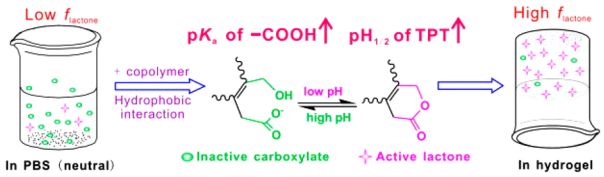


Figure 14. Schematic showing a thermoreversible hydrogel composed of copolymer PLGA–PEG–PLGA, which was was found to enhance the equilibrium fraction of the lactone form of topotecan via elevation of the carboxylate pK_a because of drug–material interactions. Reproduced from Chang et al.[68b] Copyright 2011 Elsevier.

be released from themogels in a sustained manner. In addition, cisplatin analogue Pt(IV) prodrug cisplatin can be released from polymer–platinum(IV) conjugate Bi(mPEG–PLA)–Pt-(IV) over two months in vitro.[72]

3.3. To Solve the Problem of Initial Burst Release. Thermosensitive gels present other challenges in drug release applications: initial burst release. The main reasons are (1) a solid gel is not formed immediately upon injection into the body; (2) a drug with high hydrophilicity is trapped in aqueous phase of the gel, and may diffuse into body fluid uncontrollably fast before and after thermogelation. In addition, the burst release may lead to systemic toxicity due to high dosage of drug released. Therefore, the copolymer or the drug should be designed to solve this problem. A ReGel (PLGA–PEG–PLGA) study for type 2 diabetes mellitus incorporated crystallized GLP-1 (an incretin hormone glucagon-like peptide-1 (GLP-1) was crystallized at the presence of zinc acetate). This stabilizes GLP-1 against aggregation and slows down its release.[73] The GLP-1 released from ReGel formulation in vitro and in vivo showed constant release for 2 weeks without initial burst release. Bovine serum albumin formulated in PAL–PLX–PAL thermogel was released over 1 month (in vitro) without burst release using preset-gel injection method.[60] The thermogelation takes place in the syringe for 2 min at 37 °C before injection. Recently, an injectable in situ forming gel named PME consisting of phospholipids, medium chain triglycerides (MCTs), and ethanol was developed.[74] PME remained in sol state with low viscosity in vitro and turned into a solid or semisolid gel in situ after injection, by solvent exchange method. In vitro and in vivo doxorubicin release from PME was performed and initial burst effect was hardly observed from the PME system due to fast gelation. Doxorubicin-loaded PME showed antiproliferative efficacy against MCF-7 breast cancer cells for over 5 days (Figure 15). The in vivo antitumor activities were evaluated in Kunming mice (male, 22 ± 2 g) with xenograft S180 sarcoma tumors. The sustained release of Dox from PME in tumors was maintained for more than 14 days after one single injection. Hence, this system can be used for localized chemotherapy.

4. In situ Themogels for Tissue Engineering and Other Bioapplications. Tissue engineering (TE) and regenerative medicine are rapidly developing interdisciplinary fields. Their aim is to develop biofunctional substitutes to replace or restore damaged tissues caused by chronic disease or acute trauma. Tissue engineering involves three basic components: cells, scaffolds and biomolecules. The major challenge for TE is to develop a suitable scaffold that mimics the structure and biofunctions of the native extracellular matrix (ECM). This scaffold should provide mechanical, spatial and biological signals for regulating and guiding cell growth and tissue regeneration. Hydrogels with many advanced properties

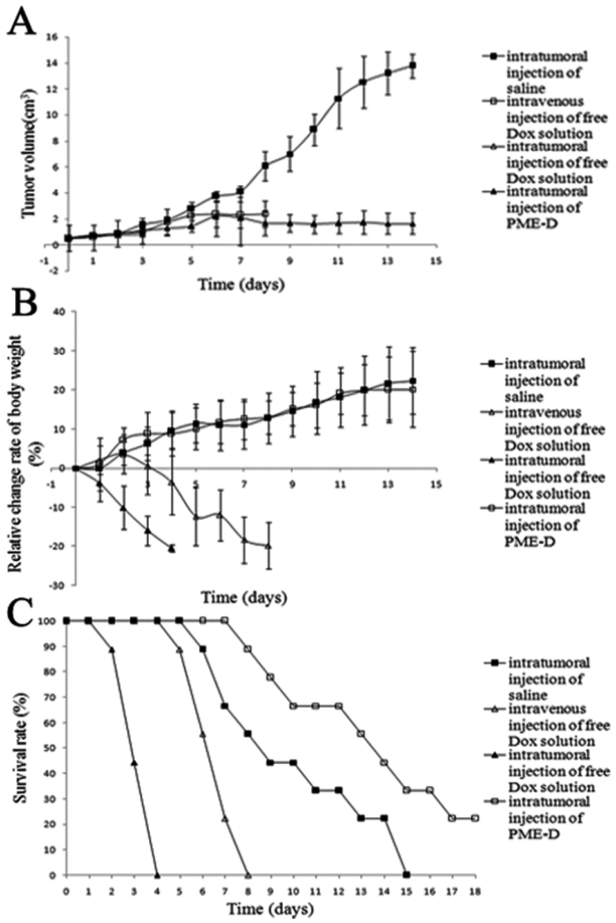


Figure 15. In vivo antitumor activity in mice bearing S180 sarcoma cancer cell xenografts. The changes in (A) tumor volume, (B) relative body weight, and (C) survival rate ($p < 0.001$) were monitored to evaluate antitumor activity. Data are represented as the mean ± standard deviation (SD) ($n = 9$). Reproduced with permission from ref 74. Copyright 2014 American Chemical Society.

such as viscoelastic nature, high water content mimicking ECM (70–80%), and amenability to chemical and physical modification, are highly attractive for biomedical scaffold design.[75] Unlike traditional 2D polystyrene culture plates, hydrogels can provide 3D living environments for cells, resulting in different morphologies and cell expression of genes and proteins. The injectability of minimally invasive in situ gelling systems is an essential consideration in the rational design of TE scaffolds. These gels allow direct injection to a specific location and are able to conform to any desired shape. Grafted cells together with functional bioingredients can be easily suspended in the in situ gelling polymer aqueous solution prior to injection. The hydrogels provide an aqueous 3D network matrix allowing for cell attachment, proliferation, migration, and even differentiation. By adjusting polymer structure, molecular weight, or concentration, the gel stiffness can also be tuned to favor mechano-transduction-mediated tissue remodelling and regeneration. However, ethanol may cause denaturation of some drugs such as proteins and peptides; thus, this system is not suitable for the delivery of these drugs.

4.1. Cardiac Tissue Engineering. As adult cardiomyocytes lack regeneration capacity, heart failure is currently incurable and leads to high morbidity and mortality worldwide.[76]

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295–316

Recently, in situ forming hydrogels have emerged as a potential biomaterial candidate to treat complex myocardial infarction (MI) for cardiac tissue regeneration. Wang et al. injected thermosensitive hydrogels containing dextran chain grafted with PCL-(2-hydroxylethyl methacrylate) (HEMA) and PNIPAAm into infarcted myocardium to replace damaged ECM in rabbits.[77] Thirty days after implantation, scar expansion and wall thinning were prevented and cardiac functions (such as left ventricular (LV) ejection fraction) were improved. Fujimoto et al. prepared a thermoresponsive hydrogel based on copolymerization of *N*-isopropylacrylamide (NIPAAm), acrylic acid (AAc) and PTMC-HEMA for the treatment of chronic infarcted myocardium.[78] The biodegradable hydrogel was injected into the infarcted LV wall in a rat chronic infarction model. Compared to the control group administered with phosphate buffered saline (PBS), a thicker LV wall and higher capillary density together with tissue ingrowth were observed at the injection site. Injectable and thermosensitive hydrogels based on PCL, NIPAAm, HEMA and dimethyl-γ-butyrolactone acrylate have also been synthesized by atom transfer radical polymerization.[79] At body temperature, the hydrogel solutions were able to form solid gels within 5s and they directed cardiogenic differentiation of cardiosphere-derived cells. Upon investigating hydrogels with different stiffness (5 kPa, 31 kPa, 65 kPa), the 31 kPa gel was found to significantly promote cardiac expression indicating that cell differentiation is affected by mechanical properties of hydrogels. The same group later reported another in situ gelling system consisting of NIPAAm, *N*-acryloxysuccinimide, AA and PTMC-HEMA for the cardiac differentiation of human mesenchymal stem cells (MSCs).[80] In the study, hydrogels with different stiffness (16, 45, and 65 kPa) showed similar cell survival ratios but different cell differentiation efficiencies. After 2 weeks of culture, MSCs in 65 kPa gels showed the highest differentiation efficiency with developed calcium channels and gap junctions for cell−cell interactions.

Incorporation of functional bioingredients into hydrogels has been demonstrated to be an effective approach in cardiac cell regeneration. A thermoresponsive amphiphilic hydrogel was synthesized for the delivery of vascular endothelial growth factor (VEGF) plasmid into hearts damaged by MI.[81] Up to 4-fold change in gene expression was observed using hydrogel-based gene transfer as compared with the naked plasmid method. The injection of VEGF-loaded plasmid hydrogels enhanced and sustained VEGF expression, and further increased capillary density and larger vessel formation in the infarcted area. To provide localized and sustained VEGF function and investigate its effects on cardiac recovery, a temperature-sensitive, aliphatic polyester hydrogel conjugated with VEGF was prepared.[82] After 35 days of implantation, the VEGF/hydrogel group enhanced blood vessel density, attenuated adverse cardiac remodelling, and improved ventricular function in a rat MI model.

Basic fibroblast growth factor (bFGF) has also been incorporated into PNIPAAm-based hydrogels for cardiac tissue engineering. These bFGF/hydrogel systems improved angiogenesis and enhanced the cardiac differentiation of MSCs under ischemic conditions.[83] A novel injectable thermosensitive hydrogel consisting of a copolymer with *N*-isopropylacrylamide/acrylic acid/2-hydroxylethyl methacrylate-poly(epsilon-caprolactone) bioconjugated with type I collagen enhanced the survival of the grafted MSCs in the myocardium. This led to enhanced neovascularization, decreased interstitial fibrosis, and thus enhanced heart function.[84] A thermosensitive chitosan chloride-glutathione (CSCl-GSH) hydrogel was prepared by conjugating glutathione on chitosan chloride.[85] These CSCl-GSH hydrogels not only showed excellent biocompatibility to support cardiomyocyte adhesion and survival, but more importantly scavenged the superoxide anion, hydroxyl, and DPPH radicals. This removal of excessive intracellular reactive oxygen species (ROS) suppresses oxidative stress damage and cardio-myocyte apoptosis.

Injectable hydrogels that are electrically conductive supports electrical stimulation of cell-tissue constructs and regulates the growth of cardiac-myocytes.[76b,d] Carbon nanotubes (CNTs) possess good mechanical strength and electrical conductivity, and hence can be utilized as additives to allow cell encapsulation and improve cardiac electrophysiological functions. Multiwalled CNTs (MWCNTs) were interpenetrated into PNIPAAm hydrogel to prepare cell sheets for cardiac tissue engineering.[34] Cell sheets of epithelial Madin-Darby canine kidney (MDCK) cells could only be harvested from PNIPAAm/MWCNTs hydrogels because of the high cell attachment ratio on the substrate. In another study, a modified PNIPAAm hydrogel was prepared by incorporating single-wall CNTs (SWCNTs) into PNIPAAm hydrogel.[86] As a carrier for intramyocardial delivery of brown adipose-derived stem cells (BASCs) after MI, the PNIPAAm/SWCNTs hydrogel significantly enhanced the engraftment of seeded cells and augmented cardiac function (Figure 16). Highly biocompatible tetraaniline (TA) is another electroactive material which has been incorporated into thermosensitive PNIPAAm-based copolymers.[87] These electroactive and thermosensitive hydrogels were found to promote the proliferation and intracellular calcium transients of H9c2 cells.

4.2. Cartilage Tissue Engineering. As a connective tissue without any neural, lymphatic or vascular supply, cartilage is notoriously difficult to be regenerated or reconstructed. Similar to the highly aqueous environment of a cartilage tissue, hydrogels are considered suitable for use in cartilage regeneration. Since the ECM of cartilage is mainly composed of proteoglycans and collagen, natural polymer based hydrogels have become the first choice in cartilage tissue engineering. Chitosan is a biocompatible polysaccharide with both hydroxyl and amino groups, which can be chemically modified easily. Thermosensitive chitosan-PNIPAAm copolymers were prepared by graft polymerization of NIPAAm into chitosan using ceric ammonium nitrate. The copolymer showed similar sol−gel transition properties as PNIPAAm.[88] After the injection of the thermosensitive chitosan-PNIPAAm gel with MSCs into rabbit bladder wall, chondrogenic differentiation of MSCs and cartilage formation were detected after 14 weeks of implantation. In another study, a thermosensitive chitosan-Pluronic hydrogel was synthesized by grafting Pluronic onto chitosan using EDC/NHS chemistry.[89] This thermogel has a gelling temperature of 25 °C, and a storage modulus of 10^4 Pa, which is similar to the stiffness of cartilage tissue. Cell culture studies indicated that the hydrogel could promote the proliferation of bovine chondrocytes and enhance the amount of synthesized glycosaminoglycan. RGD (Arg-Gly-Asp) was also conjugated onto the chitosan-Pluronic copolymers by coupling the carboxyl group in the peptide with the residual amine group in the copolymers.[90] Conjugating RGD to chitosan-Pluronic hydrogels improved the viability and proliferation of bovine chondrocytes as well as ECM expression. Ding's group synthesized an amphiphilic block

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

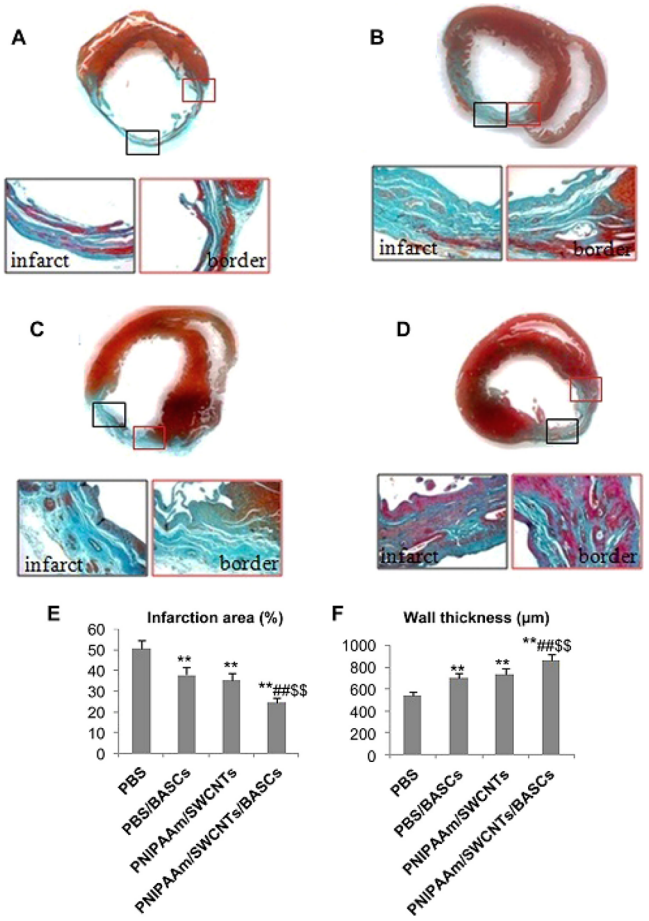


Figure 16. Infarct size and wall thickness. Cardiac structures in different groups as revealed by Masson trichrome staining 4 weeks after cell transplantation. (A) PBS group; (B) PBS/BASCs group; (C) PNIPAAm/SWCNTs group; (D) PNIPAAm/SWCNTs/BASCs group; (E, F) euantitative analysis of infarct size and infarct wall thickness, respectively (*p < 0.05 compared with PBS group; **p < 0.01 versus PBS group; #p < 0.05 and ##p < 0.01 compared with PBS/BASCs group; $ p < 0.05 and $$ p < 0.01 versus PNIPAAm/SWCNTs group). Reproduced with permission from ref 86. Copyright 2014 Elsevier.

copolymer, poly(ε-caprolactone-*co*-lactide)-PEG-poly(ε-caprolactone-*co*-lactide), and subsequently immobilized RGD into either hydrophobic poly(ε-caprolactone-*co*-lactide) (PCLA) blocks or hydrophilic PEG blocks.[91] They found that the block copolymer would form a sol−gel system and the transition temperature could be tuned between 26 to 40 °C. In vitro study showed that rat chondrocytes prefer to grow on the thermogel of RGD in hydrophilic blocks rather than those in hydrophobic blocks, highlighting that the influence of the immobilizing sites of RGD peptides in amphiphilic polymers on the eventual cell-binding efficacy. Park et al. encapsulated Tonsil-derived MSCs into a thermogelling system of PEG-poly(L-alanine-*co*-L-phenyl alanine) copolymers for 3D culture.[92] To induce cell differentiation, we provided the 3D culture system with media containing adipogenic, osteogenic, or chondrogenic factors. Interestingly, results showed that the stem cells preferentially underwent chondrogenesis with high expressions of type II collagen and sulfated glycosaminoglycan. The animal study of implantation of the hydrogel into the subcutaneous layer of mice also confirmed the chondrogenesis of the cells. It was suggested that the stiffness of the thermogels can provide biomechanical cues to guide the differentiation of the stem cells.

Mirahmadi et al. fabricated and incorporated silk fibers into thermosensitive chitosan/glycerophosphate hydrogels to reinforce the mechanical properties of the hydrogels.[93] The silk fiber reinforced chitosan hydrogels not only showed enhanced stiffness, but also supported the expression of chondrogenic phenotype for chondrocytes. Wan et al. introduced aminodiethoxypropane into alginate and the modified alginate was able to form hydrogel with chitosan.[94] This new injectable hydrogel was investigated for cartilage reconstruction by loading bone marrow mesenchymal stromal cells (BMSCs). After implanting BMSCs-laden hydrogel to a rabbit knee cartilage defect model for 12 weeks, higher levels of glycosaminoglycans (GAGs) and relative gene expression (aggrecan, collagen II, proteoglycans, and SOX9) were detected. Histology analysis showed that more chondrocytes, proteoglycans and GAGs were formed in the BMSCs-laden alginate-chitosan hydrogel group than in the defect group.

Besides chitosan, other polysaccharides such as alginate and hyaluronic acid (HA) have also been utilized for cartilage regeneration. A rigid-flexible block copolymer thermogel was developed by self-assembling ionic complex between (+)-charged amphiphilic copolymers ((polyalanine-PLX-polyalanine) and (−)-charged HA.[95] The temperature-sensitive sol-to-gel transition of the complex aqueous solution allowed it to encapsulate chondrocytes and provide a compatible microenvironment for the cells similar to a biomimetic 3D culture system. Moreover, it was found that the long-range nanofibrous structure of the thermogel played an important role for cell proliferation and protein expression.

Polypeptides and proteins also showed promising advancement in cartilage tissue engineering. Gelatin has been directly coupled to monocarboxylated Pluronic to synthesize a gelatin/Pluronic thermosensitive polymer for cartilage regeneration.[96] The polymer solution showed reversible sol−gel transition behavior at around 37 °C. Higher viability and proliferation of chondrocytes were observed in the gelatin/Pluronic hydrogel compared to the control Pluronic group. An in situ thermal gelling polypeptide (polyalanine−poloxamer−polyalanine block copolymer, sol−gel transition at 37 °C) was investigated for 3D culturing of chondrocyte.[97] The β-sheet structure of the polyalanine and the fibrous structure and stiffness of the hydrogel could regulate proliferation and protein expression of the encapsulated chondrocytes.[98] In addition, methoxy PEG−PCL diblock copolymers were found to have a sol−gel phase transition at body temperature. Kwon et al. investigated the potential use of a chondrocyte-loaded methoxy PEG−PCL hydrogel as an in situ-forming scaffold for cartilage regeneration.[99] After injection into mice, the hydrogel formed an interconnected pore structure to support the growth, proliferation and differentiation of the chondrocytes. The cell-loaded hydrogels induced cartilage growth over time in vivo, as determined by the histological and immunohistochemical staining of glycosaminoglycans, proteoglycans and collagen II (Figure 17).

4.3. Nerve Tissue Engineering. Trauma patients tend to suffer from nervous system damage and enabling sufficient functional recovery after long-gap peripheral nerve injury is a big challenge.[100] Injectable hydrogels, due to their unique rheometric properties similar to endogenous tissue, present themselves as promising candidates for neural regeneration. As early as 2001, Tate et al. reported the use of thermosensitive

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

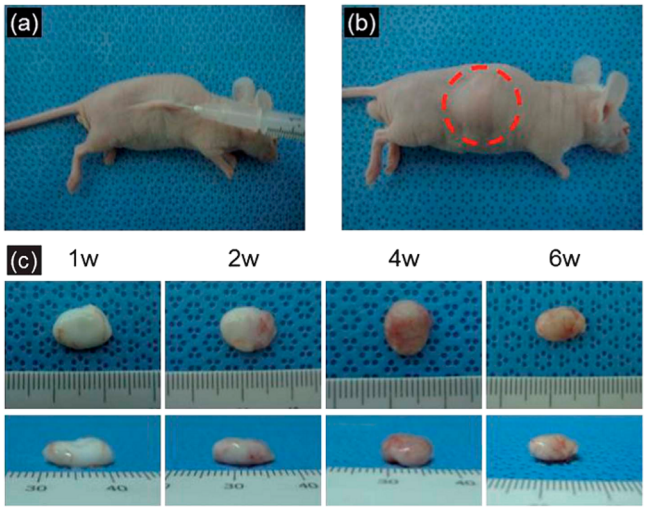


Figure 17. (a) Subcutaneous injection of chondrocyte-loaded methoxy PEG−PCL solution, (b) the formed methoxy PEG−PCL hydrogel, and (c) the hydrogels removed after 1, 2, 4, and 6 weeks. Reproduced with permission from ref 99. Copyright 2013 Royal Society of Chemistry.

methylcellulose as a scaffolding material for the treatment of brain defects.[101] The methylcellulose solutions exhibited low viscosity at room temperature (23 °C) and solidified to become a soft gel at body temperature (37 °C). In vitro cell culture study demonstrated the good biocompatibility of methylcellulose hydrogels to primary rat astrocytes or neurons. Acellular 2% methylcellulose solution was microinjected into the brains of rats 1 week after cortical impact injury, and this hydrogel assisted to limit the size of the injury cavity and the patterns of gliosis. Three different agaroses were then blended into methylcellulose separately to create thermoreversible hydrogels for nerve regeneration.[102] The agarose/methylcellulose hydrogels showed faster gelation times as compared to base methylcellulose at body temperature. Furthermore, they were able to maintain the morphology of dissociated dorsal root ganglion neurons in vitro.

Nisbet et al. investigated the potential of both thermogelling xyloglucan hydrogels and poly-D-lysine (PDL)-modified xyloglucan hydrogels for nerve tissue engineering application by implanting them within the caudate putamen of adult rats.[103] Higher concentrations of PDL in xyloglucan hydrogels led to increased infiltration levels for astrocytes and neurites. In another study, an injectable self-healing hydrogel was prepared from glycol chitosan cross-linked by telechelic difunctional PEG.[104] The self-healing hydrogel showed rapid gelation at 37 °C with a suitable stiffness of 1.5 kPa for nerve regeneration. In a zebrafish embryo neural injury model, injection of the hydrogel alone caused partial healing (~38% recovery), whereas the inclusion of neurosphere-like progenitors into the hydrogel resulted in a remarkable healing effect (~81% recovery). In another study, neuronal growth factor (and brain derived neurotropic growth factor) loaded microspheres were incorporated into a PEG-poly(L-alanine) thermogelling system for 3D cell culture.[105] Tonsil-derived MSCs were seeded in the hybrid system for neuronal differentiation. After 2 weeks of culture, the stem cells underwent multipolar elongation initially, followed by upgraded expressions of the neuronal biomarkers such as nuclear receptor related protein (Nurr-1), neuron specific enolase, microtubule associated protein-2, neurofilament-M, and glial fibrillary acidic protein in both mRNA level and protein level. The promoted neuronal differentiation of tonsil-derived MSCs was attributed to the suitable modulus of the thermogel (~800 Pa similar to the stiffness of brain tissue) and sustainable stimulation of growth factors released from the microspheres.

Collagen constitutes more than 50% of nerve ECM, and thus collagen and its derivatives have been widely evaluated as biomaterials for nerve guide applications. A nerve tissue engineering scaffold consisting of PLLA fibers and thermosensitive collagen hydrogel was designed to improve the construction of peripheral nerve.[106] Instead of using a static culture, dynamic culture was performed for bone marrow MSCs at an oscillating frequency of 0.5 Hz and 35° swing angle above and below the horizontal plane. The thermosensitive collagen hydrogel under dynamic culture enhanced the viability of the grafted cells and minimized cell loss during the initial implantation stage. Cheng et al. developed a thermosensitive gelatin/chitosan/glycerol phosphate hydrogel as a cell carrier for nucleus pulposus (NP) regeneration.[107] To overcome cell death caused by oxidative stress, we added ferulic acid (a Chinese herb medicine) into the hydrogel system for antioxidant and anti-inflammatory properties. Nucleus pulposus cells were submitted to oxidative stress caused by H_2O_2 treatment. Cells cultured on the FA-containing hydrogel exhibited down regulation of MMP-3 and hence apoptosis inhibition. Moreover, the thermosensitive hydrogel promoted NP regeneration by up-regulating mRNA levels of aggrecan and type II collagen, as well as increasing the production of sulfated-glycosaminoglycan.

4.4. Thermogels for Other Bioapplications. Postoperative intestinal adhesion is a common complication in surgery, and thermoreversible hydrogels, with biodegradable properties and unique sol−gel transition capability, could serve as a promising antiadhesion material. A thermogel system of PCLA−PEG−PCLA block copolymers was employed as a barrier material for prevention of postoperative intestinal adhesion in a rabbit model.[108] The hydrogel showed good biodegradability in vivo and its integrity could be retained for as long as several weeks. The in vivo study also proved that the thermogel system effectively reduced the formation of intraperitoneal postoperative intestinal adhesion even after 30 days. It was also reported that loading RGD molecules into PCLA−PEG−PCLA thermogel could result in a better performance in antiadhesion properties.[109] In the system, the hydrogel afforded a physical barrier and the encapsulated RGD acted as an integrin blocker to enhance the antiadhesion. Similarly, a PLGA−PEG−PLGA thermogel was synthesized as a barrier to prevent spinal epidural fibrosis in a postlaminectomy rat model.[110] Results showed that the thermogel effectively reduced epidural scarring, and prevented the subsequent adhesion to the dura mater with improved performance as compared to the positive control, chitosan gel. Yu et al. investigated the efficacy of three different PEG/polyester thermogels [PLGA−PEG−PLGA, PCL−PEG−PCL and poly(ε-caprolactone-*co*-D,L-lactic acid)-PEG-poly(ε-caprolactone-*co*-D,L-lactic acid) (PCGA−PEG−PCGA)) for preventing postoperative abdominal adhesion in a rabbit model of sidewall defect-bowel abrasion.[111] They found that PLGA−PEG−PLGA showed the best prevention of abdominal adhesions, probably due to their suitable viscoelastic properties (phase angle ≈ 45°) and excellent biodegradable rate.

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

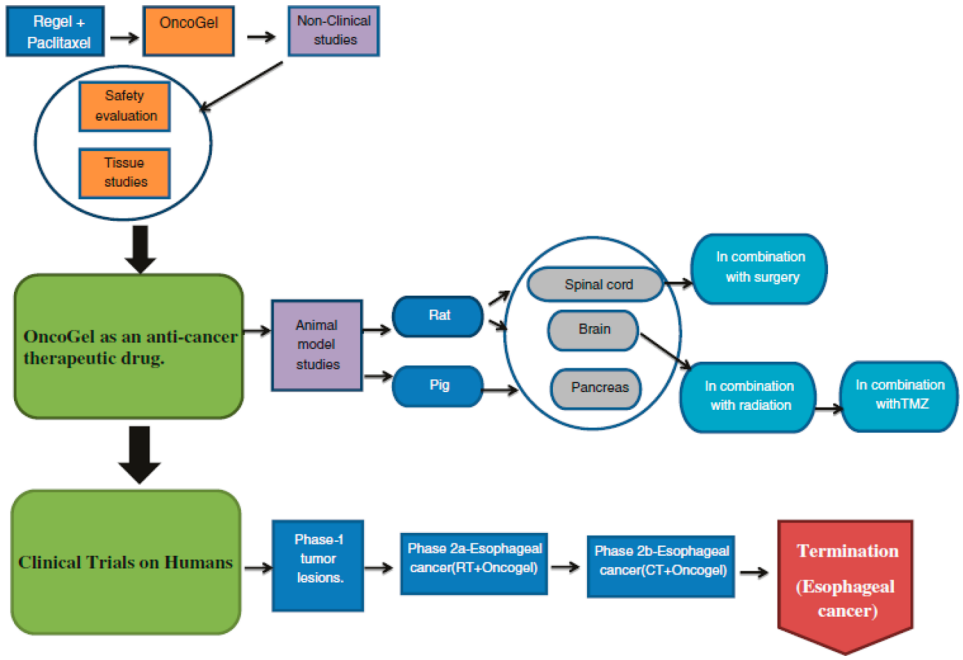


Figure 18. Flowchart of the development of OncoGel. Reproduced with permission from ref 122. Copyright 2015 Springer.

Jeong's group investigated the hepatogenic differentiation of tonsil-derived MSCs by using the 3D thermogelling matrixes of PEG-poly(L-alanine) diblock polymer.[112] The 6 wt % thermogel showed the modulus of ~1000 Pa at body temperature, which is similar to the stiffness of the decellularized liver tissue. Cocultured with hepatogenic growth factors for 4 weeks, the stem cells expressed hepatogenic genes (such as albumin, cytokeratin 18 and hepatocyte nuclear factor 4α) and exhibited typical metabolic behavior of hepatocytes (such as uptake of cardiogreen and low-density lipoprotein). An interesting work involving the use of an injectable PLGA−PEG−PLGA triblock copolymer thermogel system for sustained intravitreal delivery of dexamethasone was conducted.[113] Compared to a dexamethasone suspension, the thermogelling systems showed increased intravitreal retention time from hours to over 1 week. Moreover, the implantation of the thermogels did not impair the morphology of retina and cornea.

5. Lessons from the development of OncoGel: From Initial Concept to Clinical Trials. Limitations associated with systemic administration spurred the development of localized/targeted delivery vehicle. One such example is OncoGel, an experimental localized drug delivery system that allows the controlled release of paclitaxel (an approved intravenous anticancer drug) from thermosensitive ReGel polymer. The development of OncoGel evolved from the conception of reverse thermal gelation of biodegradable polymers consisting of A and B blocks arranged as BAB or ABA. Block A is poly(lactide-*co*-glycolide) (PLGA) and block B is poly(ethylene glycol) (PEG).[114] In 2001, MacroMed Inc. (Salt Lake City, Utah, USA) developed a thermal gel depot-based delivery system − ReGel.[115] This ABA-type biodegradable thermogel demonstrates reverse thermal gelation property where a water-soluble aqueous solution of the polymer spontaneously transforms to a water-insoluble gel at body temperature. This brought about the development of OncoGel (ReGel/paclitaxel). Upon intratumoral injection, the slow continuous release of paclitaxel into tumor tissues from ReGel minimizes systemic drug exposure and maintains therapeutic drug levels longer.[116] Both in vivo and in vitro release studies demonstrated the biocompatibility of OncoGel and the continuous release of paclitaxel concurrent with degradation over 4−6 weeks.[115,117] Clinical studies on esophageal carcinoma patients with superficially palpable tumors confirmed that OncoGel can be a stand-alone treatment or a component in combination therapies.[118] OncoGel is a promising alternative as an injectable and long-term drug delivery gel depot for cancer treatment.

5.1. Development of ReGel Polymer and OncoGel (ReGel/Paclitaxel). The need for injectable and controlled delivery of protein and poorly soluble drug molecules brought about the study of thermoresponsive PLGA/PEG-based copolymers.[16,114a,119] These hydrophilic copolymers form a free-flowing solution at room temperature but readily transforms into a hydrophobic gel at body temperature (37 °C). ReGel, a triblock copolymer with the basic structure PLGA−PEG−PLGA, fully biodegrades within one month.[115] ReGel is able to transition from a water-soluble, low viscosity solution at temperatures 2−15 °C into a water-insoluble, viscous, hydrophobically bonded gel at body temperature of 37 °C. An increase in viscosity of approximately 4 orders of magnitude accompanies the sol−gel transition. The sol−gel transition increases the solubility of hydrophobic drug by orders of magnitude (400 to >2000-fold), due to the expulsion of water molecules. The formed gel serves as an excellent drug depot. OncoGel minimizes toxicity associated with conventional systemic paclitaxel delivery through local administration and targeted cytotoxicity in solid tumors.[120] Paclitaxel exhibits anticancer activity through a number of mechanisms: mitotic inhibitor (microtubule stabilization), antiangiogenic agent, and radiation sensitizer.[121] Prasad et al. has summarized the development of OncoGel in a flowchart (Figure 18).[122]

5.2. Mechanistic Principles on Nonclinical Safety and Distribution Efficacy. OncoGel provides prolonged controlled release of paclitaxel within the tumor and physically targets the cancer site, segregating it from normal tissue.[117a,118] The release

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

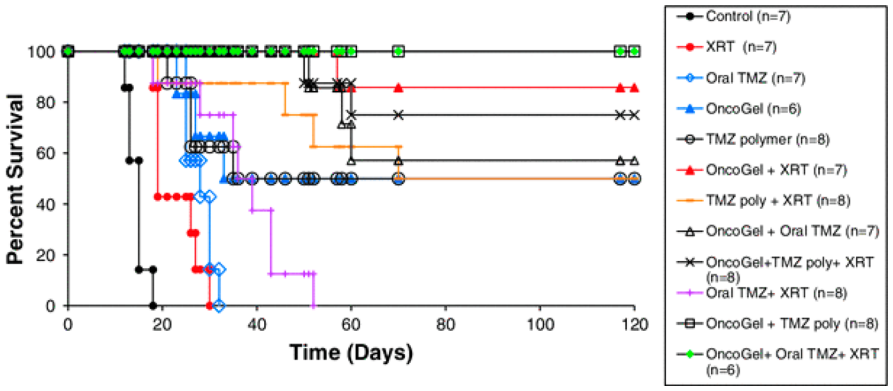


Figure 19. Intracranial efficacy of TMZ given IC or PO in combination with OncoGel 6.3 mg/mL with and without XRT for the treatment of experimental malignant gliosarcoma. F344 rats were intracranially implanted with 9L tumor. Controls (n = 7) received no further treatment (black filled circle) and had a MS of 15 days. Animals receiving XRT (20 Gy) on Day 5 (n = 7) (red filled circle) and animals that received oral TMZ on Days 5−9 (n = 7) (blue open diamond) had MS of 19 and 28 days, respectively. Animals receiving OncoGel 6.3 on Day 0 (n = 6) (blue filled triangle) had a MS of 33 days. Animals receiving a TMZ polymer on Day 5 (n = 8) (black open circle) had a MS of 35 days. Animals that received OncoGel 6.3 and XRT (n = 7) (ref filled triangle) did not reach MS with 85% long-term survivors (LTS). Animals that received a TMZ polymer and XRT (n = 8) (orange dashed line) reached MS on Day 70 with 50% LTS. Animals that received OncoGel6.3 and Oral TMZ (n = 7) (black open triangle) did not reach MS with 57% LTS. Animals receiving OncoGel 6.3, TMZ polymer and XRT (n = 8) (black cross) did not reach MS with 75% LTS. Animals that received Oral TMZ and XRT (n = 8) (magenta vertical line) had a MS of 35 days. Animals that received either OncoGel 6.3 and TMZ polymer (n = 8) (black open square) or the triple combination of OncoGel 6.3, oral TMZ and XRT (n = 6) (green filled circle) had no deaths with both groups having 100% LTS. Reproduced from Vellimana et al.[126]

profile of paclitaxel dissolved in 23% (w/w) aqueous solution of ReGel showed excellent sustained drug release over 50 days vs complete release from F-127 in approximately 1 day.[115] In tumor cells, paclitaxel binds to tubulin, inhibits the disassembly assembly dynamics of microtubules, which then induces G2/M cell cycle arrest (a relatively radiosensitive phase of the cell cycle) and cell death.[121a] Safety of OncoGel on normal tissue was conducted in rat, dog and pig models by administration into tissues: skin (subcutaneous tissue), central nervous system (both intracranial and spinal cord) and the pancreas.[115,117a,b,123] An ADME study (absorption, distribution, metabolism, and excretion) of ^{C14}paclitaxel on breast tumor xenograft in mice over 42 days following OncoGel administration intralesionally showed ^{C14}paclitaxel localized within the tumor, with minimal levels (<0.2%) detected in blood, tissues, or urine.[115] Efficacy of OncoGel (6 mg/mL paclitaxel) against human tumor xenograft (MDA231) is comparable with the maximum tolerated systemic dose (10-fold higher) of the commercial paclitaxel product, Taxol. OncoGel treatment groups exhibited no adverse effect, whereas systemic treatment groups showed weight loss and two occurrence of acute toxic death within 2 days of dosing.[115]

5.3. Development of OncoGel: Animal Model Studies to Human Clinical Trials. Preliminary mechanistic principles on safety and distribution efficacy guided the development of animal model studies. OncoGel can be used as stand-alone treatment or in combination with other known effective chemotherapy treatments which synergistically target different pathways. Efficacy studies of OncoGel on rats showed no evidence of toxicity to the spinal cord, thus delaying the onset of paresis and increasing their life-span.[117a] An endoscopic ultrasound (EUS) guided injection of OncoGel performed on porcine model showed a stable depot of OncoGel with no report of its extravasation out of the pancreas. Other observations were localized fibrotic tissue changes over 14 days and a decrease in inflammation.[123b] Efficacy studies of OncoGel combined with surgery and radiotherapy in a spinal column metastases model reported that surgery plus external beam radiotherapy (XRT) plus OncoGel resulted in a higher median BBB (Basso-Beattie-Bresnahan) score (21 vs 19, P < 0.001) than surgery plus XRT only.[117b] Studies combining OncoGel with radiotherapy on rats with intracranial 9L glioma reported prolonged median survival and increased functional motor scores. Safe doses of up to 6.3 mg/mL can be used.[117c,124] A study on OncoGel plus Temozolomide (TMZ) was shown to improve survival in patients with glioblastoma.[125] Efficacy of OncoGel plus TMZ plus RT resulted in 100% long-term survival, indicating strong therapeutic effect (Figure 19).[126]

These studies revealed that OncoGel used as adjuvant treatment prior to surgery may provide tumor shrinkage, or if used after surgery may slow or prevent tumor regrowth. This gave the impetus for evaluating OncoGel in clinical trials. Phase 1 study characterized the toxicity, pharmacokinetics, and preliminary antitumor activity associated with OncoGel administered directly into solid tumors.[118b] OncoGel delivered intralesionally at doses up to 2.0 mg paclitaxel/cm^3 tumor volume was well-tolerated and the paclitaxel remained localized at the injection site. Systemic exposure of the drug was minimized. Phase 2a was a dose-escalation study evaluating the toxicity, pharmacokinetics and preliminary antitumor activity of OncoGel plus RT therapy in patients with inoperable esophageal cancer.[118a] OncoGel given as an adjunct to RT was well tolerated in patients with inoperable esophageal cancer and provided prolonged paclitaxel release with minimal systemic exposure.[118a] OncoGel plus RT seemed to reduce tumor burden as evidenced by dysphagia improvement, tumor size reduction, and negative esophageal biopsies. These promising data spurred clinical development. Phase 2b clinical trial was conducted by combining OncoGel and chemo-radiotherapy for 154 randomized esophageal cancer patients subsequently undergoing surgery, OncoGel/Chemoradiotherapy (CRT) (n = 78) or CRT alone (n = 76).[127] Combination of intratumoral OncoGel injection and CRT was well tolerated, without a notable increase in systemic side effects. However, OncoGel failed to demonstrate enhancement in efficacy of

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

Table 2. Summary of FDA Approved Targeted Cancer Therapies[129]

target	FDA approved treatments
adenocarcinoma of the stomach or gastroesophageal junction	trastuzumab (Herceptin), ramucirumab (Cyramza)
basal cell carcinoma	vismodegib (Erivedge), sonidegib (Odomzo)
brain cancer	bevacizumab (Avastin), everolimus (Afinitor)
breast cancer	everolimus (Afinitor), tamoxifen, toremifene (Fareston), trastuzumab (Herceptin), fulvestrant (Faslodex), anastrozole (Arimidex), exemestane (Aromasin), lapatinib (Tykerb), letrozole (Femara), pertuzumab (Perjeta), ado-trastuzumabemtansine (Kadcyla), palbociclib (Ibrance)
cervical cancer	bevacizumab (Avastin)
colorectal cancer	cetuximab (Erbitux), panitumumab (Vectibix), bevacizumab (Avastin), ziv-aflibercept (Zaltrap), regorafenib (Stivarga), ramucirumab (Cyramza)
dermatofibrosarcoma protuberans	imatinibmesylate (Gleevec)
endocrine/neuroendocrine tumors	lanreotide acetate (Somatuline Depot)
head and neck cancer	cetuximab (Erbitux)
gastrointestinal stromal tumor	imatinibmesylate (Gleevec), sunitinib (Sutent), regorafenib (Stivarga)
giant cell tumor of the bone	denosumab (Xgeva)
Kaposi sarcoma	alitretinoin (Panretin)
kidney cancer	bevacizumab (Avastin), sorafenib (Nexavar), sunitinib (Sutent), pazopanib (Votrient), temsirolimus (Torisel), everolimus (Afinitor), axitinib (Inlyta)
leukemia	tretinoin (Vesanoid), imatinibmesylate (Gleevec), dasatinib (Sprycel), nilotinib (Tasigna), bosutinib (Bosulif), rituximab (Rituxan), alemtuzumab (Campath), ofatumumab (Arzerra), obinutuzumab (Gazyva), ibrutinib (Imbruvica), idelalisib (Zydelig), blinatumomab (Blincyto)
liver cancer	sorafenib (Nexavar)
lung cancer	bevacizumab (Avastin), crizotinib (Xalkori), erlotinib (Tarceva), gefitinib (Iressa), afatinibdimaleate (Gilotrif), ceritinib (LDK378/Zykadia), ramucirumab (Cyramza), nivolumab (Opdivo)
lymphoma	ibritumomabtiuxetan (Zevalin), denileukindiftitox (Ontak), brentuximabvedotin (Adcetris), rituximab (Rituxan), vorinostat (Zolinza), romidepsin (Istodax), bexarotene (Targretin), bortezomib (Velcade), pralatrexate (Folotyn), lenaliomide (Revlimid), ibrutinib (Imbruvica), siltuximab (Sylvant), idelalisib (Zydelig), belinostat (Beleodaq)
melanoma	ipilimumab (Yervoy), vemurafenib (Zelboraf), trametinib (Mekinist), dabrafenib (Tafinlar), pembrolizumab (Keytruda), nivolumab (Opdivo)
multiple myeloma	bortezomib (Velcade), carfilzomib (Kyprolis), lenaliomide (Revlimid), pomalidomide (Pomalyst), panobinostat (Farydak)
myelodysplastic/myeloproliferative disorders	imatinibmesylate (Gleevec), ruxolitinib phosphate (Jakafi)
neuroblastoma	dinutuximab (Unituxin)
ovarian epithelial/fallopian tube/primary peritoneal cancers	bevacizumab (Avastin), olaparib (Lynparza)
pancreatic cancer	erlotinib (Tarceva), everolimus (Afinitor), sunitinib (Sutent)
prostate cancer	cabazitaxel (Jevtana), enzalutamide (Xtandi), abiraterone acetate (Zytiga), radium 223 chloride (Xofigo)
soft tissue sarcoma	pazopanib (Votrient)
systemic mastocytosis	imatinibmesylate (Gleevec)
thyroid cancer	cabozantinib (Cometriq), vandetanib (Caprelsa), sorafenib (Nexavar), lenvatinibmesylate (Lenvima)

chemoradiotherapy in localized delivery of paclitaxel compared with systemic administration. OncoGel was then terminated as a potential therapy for esophageal cancer in 2010.[128]

5.4. Comparisons with Other Commercially Available Targeted Cancer Therapies. The development of targeted therapies requires the specific identification of targets that play a key role in cancer cell growth and survival. Many FDA approved therapies as summarized in Table 2[129] include hormone therapies, signal transduction inhibitors, gene expression modulator, apoptosis inducer, angiogenesis inhibitor, immune modulator, and toxin delivery molecules.

A major limitation associated with targeted therapies is resistance from cancer cells. Resistance can occur in two ways: the target itself has mutated so that the targeted therapy no longer interacts well with it, and/or the tumor finds a new pathway to achieve tumor growth that does not depend on the target. For this reason, targeted therapies may work best in combination. A multipronged approach will minimize the possibility of resistance.

5.5. Key Lessons for the Future. The limitations and uncertainties of pharmaceutical research in complex biological systems are inherent and unavoidable. Despite multiple promising nonclinical results, one cannot assume that the system will work in clinical trials. Together with safety and efficacy studies, computational mass transport simulations should be done to understand the discrepancy between animal model and humans to investigate the effectiveness of drug delivery from hydrogel-forming polymer carriers. A simulation study on paclitaxel distribution released from OncoGel between rat and human models showed different therapeutic concentrations in the relative amount of tissue for similar penetration distances. Such model provides insights to suggest modifications that improve effectiveness of drug delivery, before progressing to clinical trials.[130] Clearer conclusions drawn for paclitaxel transport mechanism in brain tissue could be applied for other tissue modeling. Modifications on ReGel polymer can be made to achieve desired properties. A number of ReGel polymers have been developed with unique properties that allow optimization of the release characteristics in order to match the desired dosing. The in situ duration and its hydrogel properties (i.e., degradation rate, pore size, hydrophobicity) can be selected by preparing a specific ReGel polymer. In one study, poly(lactide-*co*-glycolide) (PLGA) microspheres were incorporated with ReGel, as a sustained-release system for

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

perivascular delivery of dipyridamole.[131] Dipyridamole was incorporated in PLGA microspheres dispersed within the ReGel. The use of PLGA microspheres decreased the initial burst release and extended dipyridamole release from 23 to 35 days with increasing MW of PLGA. A ReGel study for type 2 diabetes mellitus incorporated GLP-1, an incretin hormone glucagon-like peptide-1 (GLP-1), as an insoluble zinc complex. This stabilizes GLP-1 against aggregation and slows down its release.[73] The GLP-1 released from ReGel formulation in vitro and in vivo showed constant release for 2 weeks with no initial burst release. Animal study demonstrated that the plasma insulin level was increased, and the blood glucose level was controlled for 2 weeks by single injection of ReGel/ZnGLP-1 formulation. Since OncoGel can be delivered to normal pancreatic tissue using EUS-guided (endoscopic ultrasound) injection, it may be feasible to administer OncoGel to tumors that are accessible via endoscopic needles using appropriate imaging techniques.[123a] Similar visualization techniques (i.e., bronchoscope, laparoscope) could be utilized to provide suitable localization to the liver and lungs. Advances in imaging and injection technologies will continue to expand the potential sites and accuracy of application.[132] More studies can be done to include injection of human-derived tumor cells into immune-compromised animal hosts to overcome limitations of using nonhuman cell lines. The goal is to attain paclitaxel responses, which will be more relevant to the clinical setting. A more accurate recreation of the tumor microenvironment allows a more accurate understanding of drug delivery mechanism before clinical studies.[124]

The primary application for OncoGel would be for inoperable tumors. This minimally invasive OncoGel treatment is a less preferred approach than open surgery because patients do not wish to wait for gradual tumor regression over time.[133] Hence, apart from performance aspects of drug delivery systems, patients' preferences and perspectives should be noted during clinical trials.

6. Outlook and Perspectives. The numerous innovative research works cited here show a spectacular evolution of thermogel technology in recent decades. Thermogels can be effectively used for delivery and encapsulation of active ingredients such as bioactive drugs, genetic material, cells, and proteins. This administration method is very convenient and noninvasive because although the gel is fluid during mixing and injection, it undergoes a sol−gel transition at the target site under physiological conditions. Copolymerization with labile groups and end-group functionalization can provide biodegradability after the goal of an implant is accomplished. The lack of popularity of OncoGel has spurred an evaluation of therapeutic methods using patients' perspectives. The challenge with OncoGel was that most patients would rather have an open surgery than to wait for tumor size reduction via a gel release system.[133] Hence the potential application of OncoGel is changed to inoperable tumors. Identifying the right application is thus essential to the success of thermogels.

For drug delivery and tissue engineering, there are stringent requirements for in situ thermogelling materials. First, the thermogel should allow easy formulation and preparation with drugs and cells. Second, the material is nontoxic and biocompatible with the gelling site. Third, the system should allow tunable and sustainable drug release profile. The stability of thermogels under specific physiological conditions depends on the environment of the targeted organs such as pH, oxidative stress, inflammation, enzymatic effect and protein adsorption. This stability determines the delivery performance of the gel. Incorporating antioxidant moieties such as vitamin E, ferulic acid, and ascorbic acid may help to reduce oxidative stress, whereas naproxen may aid in decreasing inflammation. Degradation products should be biocompatible, and these compounds are typically metabolized and excreted from our body.

The translation of the research to scalable industrial production is important. Production should be cheap, easy, and environmentally friendly. Green synthesis with less solvent and byproducts should be considered during the material-design stage. With the thermosensitive materials having LCSTs close to body temperature, it is possible to purify or precipitate the polymers with water, instead of organic solvents, at temperatures above the LCST. In addition, a wide range of thermosensitive materials remains unexplored (Table 1).

Thermoresponsive hydrogel offers reversible sol−gel transition that facilitates easy implantation and high efficacy in drug delivery. In the near future, thermogels can be exploited for more applications such as artificial vitreous substitutes, eye-drops, wound healing patch, and skincare products. In addition, the lesson from OncoGel reminds us to not only assess the suitability of the material properties but also consider the biological environment and patients' perspective. Detailed analysis from all aspects will be a step forward to a successful thermogel platform.

AUTHOR INFORMATION

Corresponding Author

*E-mail: lohxj@imre.a-star.edu.sg.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors express gratitude to the A*STAR Personal Care Grant (Project 1325400026) for support of this project, Ms. Cally Owh for providing professional art work and proof-reading, and Dr. Tan Mein Jin for proof-reading.

REFERENCES

(1) (a) Sun, J. Y.; Zhao, X.; Illeperuma, W. R.; Chaudhuri, O.; Oh, K. H.; Mooney, D. J.; Vlassak, J. J.; Suo, Z. Highly stretchable and tough hydrogels. *Nature* **2012**, *489* (7414), 133−6. (b) Low, Z. W.; Chee, P. L.; Kai, D.; Loh, X. J. The role of hydrogen bonding in alginate/poly(acrylamide-co-dimethylacrylamide) and alginate/poly(ethylene glycol) methyl ether methacrylate-based tough hybrid hydrogels. *RSC Adv.* **2015**, *5* (71), 57678−57685.

(2) (a) Appel, E. A.; del Barrio, J.; Loh, X. J.; Scherman, O. A. Supramolecular polymeric hydrogels. *Chem. Soc. Rev.* **2012**, *41* (18), 6195−214. (b) Abdul Karim, A.; Loh, X. J. Design of a micellized alpha-cyclodextrin based supramolecular hydrogel system. *Soft Matter* **2015**, *11* (27), 5425−34. (c) Ye, H.; Owh, C.; Loh, X. J. A thixotropic polyglycerol sebacate-based supramolecular hydrogel showing UCST behavior. *RSC Adv.* **2015**, *5* (60), 48720−48728. (d) Kai, D.; Low, Z. W.; Liow, S. S.; Abdul Karim, A.; Ye, H.; Jin, G.; Li, K.; Loh, X. J. Development of Lignin Supramolecular Hydrogels with Mechanically Responsive and Self-Healing Properties. *ACS Sustainable Chem. Eng.* **2015**, *3*, 2160.

(3) Jeong, B.; Kim, S. W.; Bae, Y. H. Thermosensitive sol-gel reversible hydrogels. *Adv. Drug Delivery Rev.* **2002**, *54*, 37−51.

(4) (a) Park, M. H.; Joo, M. K.; Choi, B. G.; Jeong, B. Biodegradable Thermogels. *Acc. Chem. Res.* **2012**, *45* (3), 424−433. (b) Moon, H. J.; Ko, D. Y.; Park, M. H.; Joo, M. K.; Jeong, B. Temperature-responsive compounds as in situ gelling biomedical materials. *Chem. Soc. Rev.* **2012**, *41* (14), 4860−83. (c) Loh, X. J.; Li, J. Biodegradable

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

thermosensitive copolymer hydrogels for drug delivery. *Expert Opin. Ther. Pat.* **2007**, *17* (8), 965−977. (d) Dou, Q. Q.; Liow, S. S.; Ye, E.; Lakshminarayanan, R.; Loh, X. J. Biodegradable thermogelling polymers: working towards clinical applications. *Adv. Healthcare Mater.* **2014**, *3* (7), 977−88. (e) Klouda, L.; Mikos, A. G. Thermoresponsive hydrogels in biomedical applications. *Eur. J. Pharm. Biopharm.* **2008**, *68* (1), 34−45.

(5) (a) He, C.; Kim, S. W.; Lee, D. S. In situ gelling stimuli-sensitive block copolymer hydrogels for drug delivery. *J. Controlled Release* **2008**, *127* (3), 189−207. (b) Soga, O.; van Nostrum, C. F.; Hennink, W. E. Poly(N-(2-hydroxypropyl) Methacrylamide Mono/Di Lactate): A New Class of Biodegradable Polymers with Tuneable Thermosensitivity. *Biomacromolecules* **2004**, *5*, 818−821.

(6) Roy, D.; Brooks, W. L.; Sumerlin, B. S. New directions in thermoresponsive polymers. *Chem. Soc. Rev.* **2013**, *42* (17), 7214−43.

(7) Ward, M. A.; Georgiou, T. K. Thermoresponsive Polymers for Biomedical Applications. *Polymers* **2011**, *3* (4), 1215−1242.

(8) Eustace, D. J.; Siano, D. B.; Drake, E. N. Polymer compatibility and interpolymer association in the poly(acrylic acid)−polyacrylamide−water ternary system. *J. Appl. Polym. Sci.* **1988**, *35* (3), 707−716.

(9) Ning, J.; Kubota, K.; Li, G.; Haraguchi, K. Characteristics of zwitterionic sulfobetaine acrylamide polymer and the hydrogels prepared by free-radical polymerization and effects of physical and chemical crosslinks on the UCST. *React. Funct. Polym.* **2013**, *73* (7), 969−978.

(10) Shimada, N.; Ino, H.; Maie, K.; Nakayama, M.; Kano, A.; Maruyama, A. Ureido-derivatized polymers based on both poly(allylurea) and poly(L-citrulline) exhibit UCST-type phase transition behavior under physiologically relevant conditions. *Biomacromolecules* **2011**, *12* (10), 3418−22.

(11) Shimada, N.; Kidoaki, S.; Maruyama, A. Smart hydrogels exhibiting UCST-type volume changes under physiologically relevant conditions. *RSC Adv.* **2014**, *4* (94), 52346−52348.

(12) Boustta, M.; Colombo, P. E.; Lenglet, S.; Poujol, S.; Vert, M. Versatile UCST-based thermoresponsive hydrogels for loco-regional sustained drug delivery. *J. Controlled Release* **2014**, *174*, 1−6.

(13) (a) Shachaf, Y.; Gonen-Wadmany, M.; Seliktar, D. The biocompatibility of PluronicF127 fibrinogen-based hydrogels. *Biomaterials* **2010**, *31* (10), 2836−47. (b) Ju, C.; Sun, J.; Zi, P.; Jin, X.; Zhang, C. Thermosensitive micelles-hydrogel hybrid system based on poloxamer 407 for localized delivery of paclitaxel. *J. Pharm. Sci.* **2013**, *102* (8), 2707−17.

(14) Barreiro-Iglesias, R.; Bromberg, L.; Temchenko, M.; Hatton, T. A.; Concheiro, A.; Alvarez-Lorenzo, C. Solubilization and stabilization of camptothecin in micellar solutions of pluronic-g-poly(acrylic acid) copolymers. *J. Controlled Release* **2004**, *97* (3), 537−549.

(15) Moreno, E.; Schwartz, J.; Larraneta, E.; Nguewa, P. A.; Sanmartin, C.; Agueros, M.; Irache, J. M.; Espuelas, S. Thermosensitive hydrogels of poly(methyl vinyl ether-co-maleic anhydride) — Pluronic((R)) F127 copolymers for controlled protein release. *Int. J. Pharm.* **2014**, *459*, 1−9.

(16) Jeong, B.; Bae, Y. H.; Kim, S. W. Thermoreversible Gelation of PEG−PLGA−PEG Triblock Copolymer Aqueous Solutions. *Macromolecules* **1999**, *32* (21), 7064−7069.

(17) (a) Gong, C. Y.; Shi, S.; Dong, P. W.; Kan, B.; Guo, M. L.; Wang, X. H.; Li, X. Y.; Luo, F.; Wei, Y. Q.; Qian, Z. Y. Synthesis and Characterization of PEG-PCL-PEG Thermosensitive Hydrogel. *Int. J. Pharm.* **2009**, *365* (1−2), 89−99. (b) Hwang, M. J.; Suh, J. M.; Bae, Y. H.; Kim, S. W.; Jeong, B. Caprolactonic Poloxamer Analog: PEG-PCL-PEG. *Biomacromolecules* **2005**, *6* (2), 885−890. (c) Boffito, M.; Sirianni, P.; Di Rienzo, A. M.; Chiono, V. Thermosensitive block copolymer hydrogels based on poly(epsilon-caprolactone) and polyethylene glycol for biomedical applications: state of the art and future perspectives. *J. Biomed. Mater. Res., Part A* **2015**, *103* (3), 1276−90.

(18) Li, J.; Li, X.; Ni, X.; Leong, K. W. Synthesis and Characterization of New Biodegradable Amphiphilic Poly(ethylene oxide)-b-poly[(R)-3-hydroxy butyrate]-b-poly(ethylene oxide) Tribolck Copolymers. *Macromolecules* **2003**, *36*, 2661−2667.

(19) (a) Yu, L.; Chang, G.; Zhang, H.; Ding, J. Temperature-induced spontaneous sol-gel transitions of poly(D,L-lactic acid-co-glycolic acid)-b-poly(ethylene glycol)-b-poly(D,L-lactic acid-co-glycolic acid) triblock copolymers and their end-capped derivatives in water. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45* (6), 1122−1133. (b) Yu, L.; Ding, J. Injectable hydrogels as unique biomedical materials. *Chem. Soc. Rev.* **2008**, *37* (8), 1473−81. (c) Zentner, G. M.; Rathi, R.; Shih, C.; McRea, J. C.; Seo, M.-H.; Oh, H.; Rhee, B. G.; Mestecky, J.; Moldoveanu, Z.; Morgan, M.; Weitman, S. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J. Controlled Release* **2001**, *72*, 203−215.

(20) Yu, L.; Zhang, H.; Ding, J. A subtle end-group effect on macroscopic physical gelation of triblock copolymer aqueous solutions. *Angew. Chem., Int. Ed.* **2006**, *45* (14), 2232−5.

(21) Chang, G.; Yu, L.; Yang, Z.; Ding, J. A delicate ionizable-group effect on self-assembly and thermogelling of amphiphilic block copolymers in water. *Polymer* **2009**, *50* (25), 6111−6120.

(22) Zhang, H.; Yu, L.; Ding, J. Roles of Hydrophilic Homopolymers on the Hydrophobic-Association-Induced Physical Gelling of Amphiphilic Block Copolymers in Water. *Macromolecules* **2008**, *41*, 6493−6499.

(23) (a) Chen, L.; Ci, T.; Li, T.; Yu, L.; Ding, J. Effects of Molecular Weight Distribution of Amphiphilic Block Copolymers on Their Solubility, Micellization, and Temperature-Induced Sol−Gel Transition in Water. *Macromolecules* **2014**, *47* (17), 5895−5903. (b) Chen, L.; Ci, T.; Yu, L.; Ding, J. Effects of Molecular Weight and Its Distribution of PEG Block on Micellization and Thermogellability of PLGA−PEG−PLGA Copolymer Aqueous Solutions. *Macromolecules* **2015**, *48* (11), 3662−3671.

(24) (a) Yu, L.; Zhang, Z.; Zhang, H.; Ding, J. Biodegradabiligy aand Biocompatibility of Thermoreversible Hydrogels Formed from Mixing a Sol and a Precipitate of Block Copolymers in Water. *Biomacromolecules* **2010**, *11*, 2169−2178. (b) Yu, L.; Zhang, Z.; Zhang, H.; Ding, J. Mixing a Sol and a Precipitate of Block Copolymers with Different Block Ratios Leads to an Injectable Hydrogel. *Biomacromolecules* **2009**, *10*, 1547−1553.

(25) Jeong, Y.; Joo, M. K.; Bahk, K. H.; Choi, Y. Y.; Kim, H. T.; Kim, W. K.; Lee, H. J.; Sohn, Y. S.; Jeong, B. Enzymatically degradable temperature-sensitive polypeptide as a new in-situ gelling biomaterial. *J. Controlled Release* **2009**, *137* (1), 25−30.

(26) Yun, E. J.; Yon, B.; Joo, M. K.; Jeong, B. Cell therapy for skin wound using fibroblast encapsulated poly(ethylene glycol)-poly(L-alanine) thermogel. *Biomacromolecules* **2012**, *13* (4), 1106−11.

(27) Loh, X. J.; Cheng, L. W. I.; Li, J. Micellization and Thermogelation of Poly(ether urethane)s Comprising Poly(ethylene glycol) and Poly(propylene glycol). *Macromol. Symp.* **2010**, *296* (1), 161−169.

(28) Loh, X. J.; Colin Sng, K. B.; Li, J. Synthesis and water-swelling of thermo-responsive poly(ester urethane)s containing poly(epsilon-caprolactone), poly(ethylene glycol) and poly(propylene glycol). *Biomaterials* **2008**, *29* (22), 3185−94.

(29) Loh, X. J.; Guerin, W.; Guillaume, S. M. Sustained delivery of doxorubicin from thermogelling poly(PEG/PPG/PTMC urethane)s for effective eradication of cancer cells. *J. Mater. Chem.* **2012**, *22* (39), 21249.

(30) Loh, X. J.; Tan, Y. X.; Li, Z.; Teo, L. S.; Goh, S. H.; Li, J. Biodegradable thermogelling poly(ester urethane)s consisting of poly(lactic acid)–thermodynamics of micellization and hydrolytic degradation. *Biomaterials* **2008**, *29* (14), 2164−72.

(31) Nam Nguyen, V. P.; Kuo, N.; Loh, X. J. New biocompatible thermogelling copolymers containing ethylene-butylene segments exhibiting very low gelation concentrations. *Soft Matter* **2011**, *7* (5), 2150.

(32) Loh, X. J.; Goh, S. H.; Li, J. Biodegradable Thermogelling Poly[(R)-3-hydroxybutyrate]-based Block Copolymers: Micellization, Gelation, and Cytotoxicity and Cell Culture Studies. *J. Phys. Chem. B* **2009**, *113*, 11822−11830.

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

(33) Loh, X. J.; Goh, S. H.; Li, J. New Biodegradable thermogelling copolymers having very low gelation concentration. *Biomacromolecules* **2007**, *8*, 585−593.
(34) Chen, Y. S.; Tsou, P. C.; Lo, J. M.; Tsai, H. C.; Wang, Y. Z.; Hsiue, G. H. Poly(N-isopropylacrylamide) hydrogels with interpenetrating multiwalled carbon nanotubes for cell sheet engineering. *Biomaterials* **2013**, *34* (30), 7328−7334.
(35) Schild, H. G. Poly(N-isopropylacrylamide): experiment, theory and application. *Prog. Polym. Sci.* **1992**, *17* (2), 163−249.
(36) Matyjaszewski, K. Atom Transfer Radical Polymerization (ATRP): Current Status and Future Perspectives. *Macromolecules* **2012**, *45* (10), 4015−4039.
(37) (a) Loh, X. J.; Zhang, Z.-X.; Wu, Y.-L.; Lee, T. S.; Li, J. Synthesis of Novel Biodegradable Thermoresponsive Triblock Copolymers Based on Poly[(R)-3-hydroxybutyrate] and Poly(N-isopropylacrylamide) and Their Formation of Thermoresponsive Micelles. *Macromolecules* **2009**, *42*, 194−202. (b) Kim, Y. S.; Kadla, J. F. Preparation of a thermoresponsive lignin-based biomaterial through atom transfer radical polymerization. *Biomacromolecules* **2010**, *11* (4), 981−8. (c) Loh, X. J.; Wu, Y.-L.; Joseph Seow, W. T.; Irzuan Norimzan, M. N.; Zhang, Z.-X.; Xu, F.-J.; Kang, E.-T.; Neoh, K.-G.; Li, J. Micellization and phase transition behavior of thermosensitive poly(N-isopropylacrylamide)−poly(ε-caprolactone)−poly(N-isopropylacrylamide) triblock copolymers. *Polymer* **2008**, *49* (23), 5084−5094. (d) Li, C.; Tang, Y.; Armes, S. P.; Morris, C. J.; Rose, S. F.; Lloyd, A. W.; Lewis, A. L. Synthesis and characterization of biocompatible thermo-responsive gelators based on ABA triblock copolymers. *Biomacromolecules* **2005**, *6* (2), 994−9.
(38) Diao, B.; Zhang, Z.; Zhu, J.; Li, J. Biomass-based thermogelling copolymers consisting of lignin and grafted poly(N-isopropylacrylamide), poly(ethylene glycol), and poly(propylene glycol). *RSC Adv.* **2014**, *4* (81), 42996−43003.
(39) Zhang, L.; Wang, Q.; Lei, P.; Wang, X.; Wang, C.; Cai, L. Multiblock poly(4-vinylpyridine) and its copolymer prepared with cyclic trithiocarbonate as a reversible addition−fragmentation transfer agent. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45* (13), 2617−2623.
(40) Zhou, Y.; Jiang, K.; Song, Q.; Liu, S. Thermo-induced formation of unimolecular and multimolecular micelles from novel double hydrophilic multiblock copolymers of N,N-dimethylacrylamide and N-isopropylacrylamide. *Langmuir* **2007**, *23* (26), 13076−84.
(41) Martin, L.; Gody, G.; Perrier, S. Preparation of complex multiblock copolymers via aqueous RAFT polymerization at room temperature. *Polym. Chem.* **2015**, *6* (27), 4875−4886.
(42) Ge, Z.; Zhou, Y.; Tong, Z.; Liu, S. Thermogelling of double hydrophilic multiblock and triblock copolymers of N,N-dimethylacrylamide and N-isopropylacrylamide: chain architectural and Hofmeister effects. *Langmuir* **2011**, *27* (3), 1143−51.
(43) Lutz, J. F.; Akdemir, O.; Hoth, A. Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: is the age of poly(NIPAM) over? *J. Am. Chem. Soc.* **2006**, *128* (40), 13046−7.
(44) Ma, H.; Hyun, J.; Stiller, P.; Chilkoti, A. "Non-Fouling" Oligo(ethylene glycol) Functionalized Polymer Brushes Synthesized by Surface-Initiated Atomic Transfer Radical Polymerization. *Adv. Mater.* **2004**, *16* (4), 338−341.
(45) (a) Liu, M.; Leroux, J.-C.; Gauthier, M. A. Conformation−function relationships for the comb-shaped polymer pOEGMA. *Prog. Polym. Sci.* **2015**, *48*, 111−121. (b) Vancoillie, G.; Frank, D.; Hoogenboom, R. Thermoresponsive poly(oligo ethylene glycol acrylates). *Prog. Polym. Sci.* **2014**, *39* (6), 1074−1095.
(46) Lutz, J.-F. Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46* (11), 3459−3470.
(47) Lutz, J.-F.; Hoth, A. Preparation of Ideal PEG Analogues with a Tunable Thermosensitivity by Controlled Radical Copolymerization of 2-(2-Methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. *Macromolecules* **2006**, *39*, 893−896.
(48) http://www.fda.gov/downloads/drugs/developmentapprovalprocess/formssubmissionrequirements/drugmasterfilesdmfs/ucm370723.txt.
(49) (a) Loh, X. J.; Tan, K. K.; Li, X.; Li, J. The in vitro hydrolysis of poly(ester urethane)s consisting of poly[(R)-3-hydroxybutyrate] and poly(ethylene glycol). *Biomaterials* **2006**, *27* (9), 1841−1850. (b) Loh, X. J.; Tan, Y. X.; Li, Z.; Teo, L. S.; Goh, S. H.; Li, J. Biodegradable thermogelling poly(ester urethane)s consisting of poly(lactic acid) − Thermodynamics of micellization and hydrolytic degradation. *Biomaterials* **2008**, *29* (14), 2164−2172. (c) Joo, M. K.; Sohn, Y. S.; Jeong, B. Stereoisomeric effect on reverse thermal gelation of poly (ethylene glycol)/poly (lactide) multiblock copolymer. *Macromolecules* **2007**, *40* (14), 5111−5115. (d) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. Biodegradable block copolymers as injectable drug-delivery systems. *Nature* **1997**, *388* (6645), 860−862. (e) Bae, S. J.; Suh, J. M.; Sohn, Y. S.; Bae, Y. H.; Kim, S. W.; Jeong, B. Thermogelling poly (caprolactone-b-ethylene glycol-b-caprolactone) aqueous solutions. *Macromolecules* **2005**, *38* (12), 5260−5265.
(50) (a) Jeong, Y.; Joo, M. K.; Bahk, K. H.; Choi, Y. Y.; Kim, H.-T.; Kim, W.-K.; Lee, H. J.; Sohn, Y. S.; Jeong, B. Enzymatically degradable temperature-sensitive polypeptide as a new in-situ gelling biomaterial. *J. Controlled Release* **2009**, *137* (1), 25−30. (b) Choi, B. G.; Park, M. H.; Cho, S.-H.; Joo, M. K.; Oh, H. J.; Kim, E. H.; Park, K.; Han, D. K.; Jeong, B. In situ thermal gelling polypeptide for chondrocytes 3D culture. *Biomaterials* **2010**, *31* (35), 9266−9272. (c) Moon, H. J.; Choi, B. G.; Park, M. H.; Joo, M. K.; Jeong, B. Enzymatically degradable thermogelling poly (alanine-co-leucine)-poloxamer-poly (alanine-co-leucine). *Biomacromolecules* **2011**, *12* (4), 1234−1242. (d) Garripelli, V. K.; Kim, J.-K.; Son, S.; Kim, W. J.; Repka, M. A.; Jo, S. Matrix metalloproteinase-sensitive thermogelling polymer for bioresponsive local drug delivery. *Acta Biomater.* **2011**, *7* (5), 1984−1992.
(51) Jeong, B.; Bae, Y. H.; Kim, S. W. Drug release from biodegradable injectable thermosensitive hydrogel of PEG−PLGA−PEG triblock copolymers. *J. Controlled Release* **2000**, *63* (1−2), 155−163.
(52) Jeong, B.; Bae, Y. H.; Kim, S. W. In situ gelation of PEG−PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. *J. Biomed. Mater. Res.* **2000**, *50* (2), 171−177.
(53) Shim, W. S.; Kim, J.-H.; Park, H.; Kim, K.; Chan Kwon, I.; Lee, D. S. Biodegradability and biocompatibility of a pH- and thermo-sensitive hydrogel formed from a sulfonamide-modified poly(ε-caprolactone-co-lactide)−poly(ethylene glycol)−poly(ε-caprolactone-co-lactide) block copolymer. *Biomaterials* **2006**, *27* (30), 5178−5185.
(54) Loh, X. J.; Goh, S. H.; Li, J. Hydrolytic degradation and protein release studies of thermogelling polyurethane copolymers consisting of poly[(R)-3-hydroxybutyrate], poly(ethylene glycol), and poly-(propylene glycol). *Biomaterials* **2007**, *28* (28), 4113−23.
(55) Gopferich, A.; Langer, R. Modeling of polymer erosion. *Macromolecules* **1993**, *26* (16), 4105−4112.
(56) Chen, C. F.; Lin, C. T. Y.; Chu, I. M. Study of novel biodegradable thermo-sensitive hydrogels of methoxy-poly(ethylene glycol)-block-polyester diblock copolymers. *Polym. Int.* **2010**, *59* (10), 1428−1435.
(57) Loh, X. J.; Goh, S. H.; Li, J. Hydrolytic degradation and protein release studies of thermogelling polyurethane copolymers consisting of poly [(R)-3-hydroxybutyrate], poly (ethylene glycol), and poly (propylene glycol). *Biomaterials* **2007**, *28* (28), 4113−4123.
(58) (a) Sun, K. H.; Sohn, Y. S.; Jeong, B. Thermogelling Poly(ethylene oxide-b-propylene oxide-b-ethylene oxide Disulfide Multiblock Copolymer as aThiol-Sensitive Degradable Polymer. *Biomacromolecules* **2006**, *7*, 2871−2877. (b) Ko, D. Y.; Shinde, U. P.; Yeon, B.; Jeong, B. Recent progress of in situ formed gels for biomedical applications. *Prog. Polym. Sci.* **2013**, *38* (3−4), 672−701.
(59) Mei-Chun, L.; Kung-Chin, C.; Sheng-Chieh, H.; Ming-Chieh, C.; Wei, I. H.; You-Rong, H.; Hung-Maan, L.; Ming-Fa, H.; Jui-Ming, Y. In situ gelation of PEG−PLGA-PEG hydrogels containing high loading of hydroxyapatite: in vitro and in vivo characteristics. *Biomed. Mater.* **2014**, *9* (1), 015011.
(60) Moon, H. J.; Choi, B. G.; Park, M. H.; Joo, M. K.; Jeong, B. Enzymatically degradable thermogelling poly(alanine-co-leucine)-

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

poloxamer-poly(alanine-co-leucine). *Biomacromolecules* **2011**, *12* (4), 1234−42.
(61) Göpferich, A. Polymer bulk erosion. *Macromolecules* **1997**, *30* (9), 2598−2604.
(62) (a) Rothstein, S. N.; Federspiel, W. J.; Little, S. R. A unified mathematical model for the prediction of controlled release from surface and bulk eroding polymer matrices. *Biomaterials* **2009**, *30* (8), 1657−1664. (b) Burkersroda, F. v.; Schedl, L.; Göpferich, A. Why degradable polymers undergo surface erosion or bulk erosion. *Biomaterials* **2002**, *23* (21), 4221−4231.
(63) Potta, T.; Chun, C.; Song, S.-C. Controlling the degradation rate of thermoresponsive photo-cross-linked poly(organophosphazene) hydrogels with compositions of depsipeptide and PEG chain lengths. *Polym. Degrad. Stab.* **2011**, *96* (7), 1261−1270.
(64) Graham, P. D.; Brodbeck, K. J.; McHugh, A. J. Phase inversion dynamics of PLGA solutions related to drug delivery. *J. Controlled Release* **1999**, *58* (2), 233−245.
(65) Thakur, R. R. S.; McMillan, H. L.; Jones, D. S. Solvent induced phase inversion-based in situ forming controlled release drug delivery implants. *J. Controlled Release* **2014**, *176*, 8−23.
(66) Jeong, B.; Bae, Y. H.; Kim, S. W. Drug release from biodegradable injectable thermosensitive hydrogel of PEG−PLGA−PEG triblock copolymers. *J. Controlled Release* **2000**, *63*, 155−163.
(67) Wei, G.; Xu, H.; Ding, P. T.; Li, S. M.; Zheng, J. M. Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies. *J. Controlled Release* **2002**, *83* (1), 65−74.
(68) (a) Ci, T.; Chen, L.; Yu, L.; Ding, J. Tumor regression achieved by encapsulating a moderately soluble drug into a polymeric thermogel. *Sci. Rep.* **2014**, *4*, 5473. (b) Chang, G.; Ci, T.; Yu, L.; Ding, J. Enhancement of the fraction of the active form of an antitumor drug topotecan via an injectable hydrogel. *J. Controlled Release* **2011**, *156* (1), 21−27. (c) Yu, L.; Chang, G. T.; Zhang, H.; Ding, J. D. Injectable block copolymer hydrogels for sustained release of a PEGylated drug. *Int. J. Pharm.* **2008**, *348* (1−2), 95−106.
(69) (a) Ci, T.; Chen, L.; Li, T.; Chang, G.; Yu, L.; Ding, J. Effects of amphiphilic block copolymers on the equilibrium lactone fractions of camptothecin analogues at different pHs. *Biomater. Sci.* **2013**, *1* (12), 1235−1243. (b) Ci, T.; Li, T.; Chen, L.; Chang, G.; Yu, L.; Ding, J. Effects of ″mature micelle″ formation of Pluronic P123 on equilibrium between lactone and carboxylate forms of 10-hydrocamptothecin in water. *Polym. Chem.* **2013**, *4* (11), 3245−3255.
(70) (a) Yu, L.; Zhang, Z.; Ding, J. In vitro degradation and protein release of transparent and opaque physical hydrogels of block copolymers at body temperature. *Macromol. Res.* **2012**, *20* (3), 234−243. (b) Li, K.; Yu, L.; Liu, X.; Chen, C.; Chen, Q.; Ding, J. A long-acting formulation of a polypeptide drug exenatide in treatment of diabetes using an injectable block copolymer hydrogel. *Biomaterials* **2013**, *34* (11), 2834−2842. (c) Yu, L.; Li, K.; Liu, X.; Chen, C.; Bao, Y.; Ci, T.; Chen, Q.; Ding, J. In Vitro and In Vivo Evaluation of a Once-weekly Formulation of an Antidiabetic Peptide Drug Exenatide in an Injectable Thermogel. *J. Pharm. Sci.* **2013**, *102* (11), 4140−4149.
(71) Yu, L.; Ci, T.; Zhou, S.; Zeng, W.; Ding, J. The thermogelling PLGA-PEG−PLGA block copolymer as a sustained release matrix of doxorubicin. *Biomater. Sci.* **2013**, *1* (4), 411−420.
(72) Shen, W.; Luan, J.; Cao, L.; Sun, J.; Yu, L.; Ding, J. Thermogelling Polymer−Platinum(IV) Conjugates for Long-Term Delivery of Cisplatin. *Biomacromolecules* **2015**, *16* (1), 105−115.
(73) Choi, S.; Baudys, M.; Kim, S. W. Control of blood glucose by novel GLP-1 delivery using biodegradable triblock copolymer of PLGA-PEG−PLGA in type 2 diabetic rats. *Pharm. Res.* **2004**, *21* (5), 827−31.
(74) Wu, W.; Chen, H.; Shan, F.; Zhou, J.; Sun, X.; Zhang, L.; Gong, T. A Novel Doxorubicin-Loaded in Situ Forming Gel Based High Concentration of Phospholipid for Intratumoral Drug Delivery. *Mol. Pharmaceutics* **2014**, *11* (10), 3378−3385.
(75) Kai, D.; Prabhakaran, M. P.; Stahl, B.; Eblenkamp, M.; Wintermantel, E.; Ramakrishna, S. Mechanical properties and in vitro behavior of nanofiber-hydrogel composites for tissue engineering applications. *Nanotechnology* **2012**, *23* (9), 095705.
(76) (a) Kai, D.; Wang, Q. L.; Wang, H. J.; Prabhakaran, M. P.; Zhang, Y. Z.; Tan, Y. Z.; Ramakrishna, S. Stem cell-loaded nanofibrous patch promotes the regeneration of infarcted myocardium with functional improvement in rat model. *Acta Biomater.* **2014**, *10* (6), 2727−2738. (b) Kai, D.; Prabhakaran, M. P.; Jin, G. R.; Ramakrishna, S. Biocompatibility evaluation of electrically conductive nanofibrous scaffolds for cardiac tissue engineering. *J. Mater. Chem. B* **2013**, *1* (17), 2305−2314. (c) Kai, D.; Prabhakaran, M. P.; Jin, G. R.; Ramakrishna, S. Guided orientation of cardiomyocytes on electrospun aligned nanofibers for cardiac tissue engineering. *J. Biomed. Mater. Res., Part B* **2011**, *98B* (2), 379−386. (d) Kai, D.; Prabhakaran, M. P.; Jin, G. R.; Ramakrishna, S. Polypyrrole-contained electrospun conductive nanofibrous membranes for cardiac tissue engineering. *J. Biomed. Mater. Res., Part A* **2011**, *99A* (3), 376−385.
(77) Wang, T.; Wu, D. Q.; Jiang, X. J.; Zhang, X. Z.; Li, X. Y.; Zhang, J. F.; Zheng, Z. B.; Zhuo, R. X.; Jiang, H.; Huang, C. X. Novel thermosensitive hydrogel injection inhibits post-infarct ventricle remodelling. *Eur. J. Heart Failure* **2009**, *11* (1), 14−19.
(78) Fujimoto, K.; Ma, Z.; Nelson, D.; Hashizume, R.; Guan, J.; Tobita, K.; Wagner, W. Synthesis, characterization and therapeutic efficacy of a biodegradable, thermoresponsive hydrogel designed for application in chronic infarcted myocardium. *Biomaterials* **2009**, *30* (26), 4357−4368.
(79) Li, Z. Q.; Guo, X. L.; Matsushita, S.; Guan, J. J. Differentiation of cardiosphere-derived cells into a mature cardiac lineage using biodegradable poly(N-isopropylacrylamide) hydrogels. *Biomaterials* **2011**, *32* (12), 3220−3232.
(80) Li, Z. Q.; Guo, X. L.; Palmer, A. F.; Das, H.; Guan, J. J. High-efficiency matrix modulus-induced cardiac differentiation of human mesenchymal stem cells inside a thermosensitive hydrogel. *Acta Biomater.* **2012**, *8* (10), 3586−3595.
(81) Kwon, J. S.; Park, I. K.; Cho, A. S.; Shin, S. M.; Hong, M. H.; Jeong, S. Y.; Kim, Y. S.; Min, J. J.; Jeong, M. H.; Kim, W. J.; Jo, S.; Pun, S. H.; Cho, J. G.; Park, J. C.; Kang, J. C.; Ahn, Y. Enhanced angiogenesis mediated by vascular endothelial growth factor plasmid-loaded thermo-responsive amphiphilic polymer in a rat myocardial infarction model. *J. Controlled Release* **2009**, *138* (2), 168−176.
(82) Wu, J.; Zeng, F. Q.; Huang, X. P.; Chung, J. C. Y.; Konecny, F.; Weisel, R. D.; Li, R. K. Infarct stabilization and cardiac repair with a VEGF-conjugated, injectable hydrogel. *Biomaterials* **2011**, *32* (2), 579−586.
(83) (a) Garbern, J. C.; Minami, E.; Stayton, P. S.; Murry, C. E. Delivery of basic fibroblast growth factor with a pH-responsive, injectable hydrogel to improve angiogenesis in infarcted myocardium. *Biomaterials* **2011**, *32* (9), 2407−2416. (b) Li, Z. Q.; Guo, X. L.; Guan, J. J. A Thermosensitive Hydrogel Capable of Releasing bFGF for Enhanced Differentiation of Mesenchymal Stem Cell into Cardiomyocyte-like Cells under Ischemic Conditions. *Biomacromolecules* **2012**, *13* (6), 1956−1964.
(84) Xia, Y.; Zhu, K.; Lai, H.; Lang, M. D.; Xiao, Y.; Lian, S.; Guo, C. F.; Wang, C. S. Enhanced infarct myocardium repair mediated by thermosensitive copolymer hydrogel-based stem cell transplantation. *Exp. Biol. Med.* **2015**, *240* (5), 593−600.
(85) Li, J. J.; Shu, Y.; Hao, T.; Wang, Y.; Qian, Y. F.; Duan, C. M.; Sun, H. Y.; Lin, Q. X.; Wang, C. Y. A chitosan-glutathione based injectable hydrogel for suppression of oxidative stress damage in cardiomyocytes. *Biomaterials* **2013**, *34* (36), 9071−9081.
(86) Li, X.; Zhou, J.; Liu, Z. Q.; Chen, J.; Lu, S. H.; Sun, H. Y.; Li, J. J.; Lin, Q. X.; Yang, B. G.; Duan, C. M.; Xing, M.; Wang, C. Y. A PNIPAAm-based thermosensitive hydrogel containing SWCNTs for stem cell transplantation in myocardial repair. *Biomaterials* **2014**, *35* (22), 5679−5688.
(87) Cui, H. T.; Liu, Y. D.; Cheng, Y. L.; Zhang, Z.; Zhang, P. B.; Chen, X. S.; Wei, Y. In Vitro Study of Electroactive Tetraaniline-Containing Thermosensitive Hydrogels for Cardiac Tissue Engineering. *Biomacromolecules* **2014**, *15* (4), 1115−1123.

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

(88) Cho, J. H.; Kim, S. H.; Park, K. D.; Jung, M. C.; Yang, W. I.; Han, S. W.; Noh, J. Y.; Lee, J. W. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(N-isopropylacrylamide) and water-soluble chitosan copolymer. *Biomaterials* **2004**, *25* (26), 5743−5751.
(89) Park, K. M.; Lee, S. Y.; Joung, Y. K.; Na, J. S.; Lee, M. C.; Park, K. D. Thermosensitive chitosan-Pluronic hydrogel as an injectable cell delivery carrier for cartilage regeneration. *Acta Biomater.* **2009**, *5* (6), 1956−1965.
(90) Park, K. M.; Joung, Y. K.; Park, K. D.; Lee, S. Y.; Lee, M. C. RGD-conjugated chitosan-Pluronic hydrogels as a cell supported scaffold for articular cartilage regeneration. *Macromol. Res.* **2008**, *16* (6), 517−523.
(91) Zhang, Z.; Lai, Y. X.; Yu, L.; Ding, J. D. Effects of immobilizing sites of RGD peptides in amphiphilic block copolymers on efficacy of cell adhesion. *Biomaterials* **2010**, *31* (31), 7873−7882.
(92) Park, M. H.; Yu, Y.; Moon, H. J.; Ko, D. Y.; Kim, H. S.; Lee, H.; Ryu, K. H.; Jeong, B. 3D Culture of Tonsil-Derived Mesenchymal Stem Cells in Poly(ethylene glycol)-Poly(L-alanine-co-L-phenyl alanine) Thermogel. *Adv. Healthcare Mater.* **2014**, *3* (11), 1782−1791.
(93) Mirahmadi, F.; Tafazzoli-Shadpour, M.; Shokrgozar, M. A.; Bonakdar, S. Enhanced mechanical properties of thermosensitive chitosan hydrogel by silk fibers for cartilage tissue engineering. *Mater. Sci. Eng., C* **2013**, *33* (8), 4786−4794.
(94) Wan, W. B.; Li, Q. T.; Gao, H. Y.; Ge, L. P.; Liu, Y. Q.; Zhong, W.; Ouyang, J.; Xing, M. BMSCs laden injectable amino-diethoxypropane modified alginate-chitosan hydrogel for hyaline cartilage reconstruction. *J. Mater. Chem. B* **2015**, *3* (9), 1990−2005.
(95) Park, M. H.; Choi, B. G.; Jeong, B. Complexation-Induced Biomimetic Long Range Fibrous Orientation in a Rigid-Flexible Block Copolymer Thermogel. *Adv. Funct. Mater.* **2012**, *22* (24), 5118−5125.
(96) Kim, D. H.; Heo, S. J.; Shin, J. W.; Mun, C. W.; Park, K. M.; Park, K. D.; Jee, K. S. Preparation of Thermosensitive Gelatin-Pluronic Copolymer for Cartilage Tissue Engineering. *Macromol. Res.* **2010**, *18* (4), 387−391.
(97) Choi, B. G.; Park, M. H.; Cho, S. H.; Joo, M. K.; Oh, H. J.; Kim, E. H.; Park, K.; Han, D. K.; Jeong, B. In situ thermal gelling polypeptide for chondrocytes 3D culture. *Biomaterials* **2010**, *31* (35), 9266−9272.
(98) Choi, B. G.; Park, M. H.; Cho, S. H.; Joo, M. K.; Oh, H. J.; Kim, E. H.; Park, K.; Han, D. K.; Jeong, B. Thermal gelling polyalanine-poloxamine-polyalanine aqueous solution for chondrocytes 3D culture: Initial concentration effect. *Soft Matter* **2011**, *7* (2), 456−462.
(99) Kwon, J. S.; Yoon, S. M.; Kwon, D. Y.; Kim, D. Y.; Tai, G. Z.; Jin, L. M.; Song, B.; Lee, B.; Kim, J. H.; Han, D. K.; Min, B. H.; Kim, M. S. Injectable in situ-forming hydrogel for cartilage tissue engineering. *J. Mater. Chem. B* **2013**, *1* (26), 3314−3321.
(100) Lin, Y. C.; Marra, K. G. Injectable systems and implantable conduits for peripheral nerve repair. *Biomed. Mater.* **2012**, *7* (2), 024102.
(101) Tate, M. C.; Shear, D. A.; Hoffman, S. W.; Stein, D. G.; LaPlaca, M. C. Biocompatibility of methylcellulose-based constructs designed for intracerebral gelation following experimental traumatic brain injury. *Biomaterials* **2001**, *22* (10), 1113−1123.
(102) Martin, B. C.; Minner, E. J.; Wiseman, S. L.; Klank, R. L.; Gilbert, R. J. Agarose and methylcellulose hydrogel blends for nerve regeneration applications. *J. Neural Eng.* **2008**, *5* (2), 221−231.
(103) Nisbet, D. R.; Rodda, A. E.; Horne, M. K.; Forsythe, J. S.; Finkelstein, D. I. Implantation of Functionalized Thermally Gelling Xyloglucan Hydrogel Within the Brain: Associated Neurite Infiltration and Inflammatory Response. *Tissue Eng., Part A* **2010**, *16* (9), 2833−2842.
(104) Tseng, T. C.; Tao, L.; Hsieh, F. Y.; Wei, Y.; Chiu, I. M.; Hsu, S. H. An Injectable, Self-Healing Hydrogel to Repair the Central Nervous System. *Adv. Mater.* **2015**, *27* (23), 3518−3524.
(105) Patel, M.; Moon, H. J.; Jung, B. K.; Jeong, B. Microsphere-Incorporated Hybrid Thermogel for Neuronal Differentiation of Tonsil Derived Mesenchymal Stem Cells. *Adv. Healthcare Mater.* **2015**, *4* (10), 1565−1574.
(106) Huang, L. F.; Li, R.; Liu, W. G.; Dai, J.; Du, Z. W.; Wang, X. N.; Ma, J. C.; Zhao, J. S. Dynamic culture of a thermosensitive collagen hydrogel as an extracellular matrix improves the construction of tissue-engineered peripheral nerve. *Neural Regener. Res.* **2014**, *9* (14), 1371−1378.
(107) Cheng, Y. H.; Yang, S. H.; Liu, C. C.; Gefen, A.; Lin, F. H. Thermosensitive hydrogel made of ferulic acid-gelatin and chitosan glycerophosphate. *Carbohydr. Polym.* **2013**, *92* (2), 1512−1519.
(108) Zhang, Z.; Ni, J.; Chen, L.; Yu, L.; Xu, J. W.; Ding, J. D. Biodegradable and thermoreversible PCLA-PEG-PCLA hydrogel as a barrier for prevention of post-operative adhesion. *Biomaterials* **2011**, *32* (21), 4725−4736.
(109) Zhang, Z.; Ni, J.; Chen, L.; Yu, L.; Xu, J. W.; Ding, J. D. Encapsulation of cell-adhesive RGD peptides into a polymeric physical hydrogel to prevent postoperative tissue adhesion. *J. Biomed. Mater. Res., Part B* **2012**, *100B* (6), 1599−1609.
(110) Li, X.; Chen, L.; Lin, H.; Cao, L.; Cheng, J. a.; Dong, J.; Yu, L.; Ding, J. Efficacy of Poly(D,L-Lactic Acid-co-Glycolic acid)-Poly-(Ethylene Glycol)-Poly(D,L-Lactic Acid-co-Glycolic Acid) Thermogel As a Barrier to Prevent Spinal Epidural Fibrosis in a Postlaminectomy Rat Model. *J. Spinal Disorders Tech.* **2016**, 1.
(111) Yu, L.; Hu, H. T.; Chen, L.; Bao, X. G.; Li, Y. Z.; Xu, G. H.; Ye, X. J.; Ding, J. D. Comparative studies of thermogels in preventing post-operative adhesions and corresponding mechanisms. *Biomater. Sci.* **2014**, *2* (8), 1100−1109.
(112) Kim, S. J.; Park, M. H.; Moon, H. J.; Park, J. H.; Ko, D. Y.; Jeong, B. Polypeptide Thermogels as a Three Dimensional Culture Scaffold for Hepatogenic Differentiation of Human Tonsil-Derived Mesenchymal Stem Cells. *ACS Appl. Mater. Interfaces* **2014**, *6* (19), 17034−17043.
(113) Zhang, L.; Shen, W. J.; Luan, J. B.; Yang, D. X.; Wei, G.; Yu, L.; Lu, W. Y.; Ding, J. D. Sustained intravitreal delivery of dexamethasone using an injectable and biodegradable thermogel. *Acta Biomater.* **2015**, *23*, 271−281.
(114) (a) Choi, Y. Y.; Joo, M. K.; Sohn, Y. S.; Jeong, B. Significance of secondary structure in nanostructure formation and thermosensitivity of polypeptide block copolymers. *Soft Matter* **2008**, *4* (12), 2383. (b) Rathi, R. C.; Zentner, G. M.; Jeong, B., Biodegradable low molecular weight triblock poly (lactide-co-glycolide) polyethylene glycol copolymers having reverse thermal gelation properties. U.S. Patent 6004573 A, 2000. (c) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. Biodegradable block copolymers as injectable drug-delivery systems. *Nature* **1997**, *388* (6645), 860−862. (d) Cha, Y.; Choi, Y. K.; Bae, Y. H. Thermosensitive biodegradable polymers based on poly(ether-ester)block copolymers. U.S. Patent 5702717 A, 1997.
(115) Zentner, G. M.; Rathi, R.; Shih, C.; McRea, J. C.; Seo, M. H.; Oh, H.; Rhee, B. G.; Mestecky, J.; Moldoveanu, Z.; Morgan, M.; Weitman, S. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J. Controlled Release* **2001**, *72* (1−3), 203−15.
(116) Elstad, N. L.; Fowers, K. D. OncoGel (ReGel/paclitaxel)−clinical applications for a novel paclitaxel delivery system. *Adv. Drug Delivery Rev.* **2009**, *61* (10), 785−94.
(117) (a) Bagley, C. A.; Bookland, M. J.; Pindrik, J. A.; Ozmen, T.; Gokaslan, Z. L.; Witham, T. F. Local delivery of oncogel delays paresis in rat metastatic spinal tumor model. *Journal of neurosurgery. Spine* **2007**, *7* (2), 194−8. (b) Gok, B.; McGirt, M. J.; Sciubba, D. M.; Garces-Ambrossi, G.; Nelson, C.; Noggle, J.; Bydon, A.; Witham, T. F.; Wolinsky, J. P.; Gokaslan, Z. L. Adjuvant treatment with locally delivered OncoGel delays the onset of paresis after surgical resection of experimental spinal column metastasis. *Neurosurgery* **2009**, *65* (1), 193−1999. (c) Tyler, B.; Fowers, K. D.; Li, K. W.; Recinos, V. R.; Caplan, J. M.; Hdeib, A.; Grossman, R.; Basaldella, L.; Bekelis, K.; Pradilla, G.; Legnani, F.; Brem, H. A thermal gel depot for local delivery of paclitaxel to treat experimental brain tumors in rats. *J. Neurosurg.* **2010**, *113* (2), 210−7.
(118) (a) DuVall, G. A.; Tarabar, D.; Seidel, R. H.; Elstad, N. L.; Fowers, K. D. Phase 2: a dose-escalation study of OncoGel (ReGel/paclitaxel), a controlled-release formulation of paclitaxel, as adjunctive

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316

local therapy to external-beam radiation in patients with inoperable esophageal cancer. *Anti-Cancer Drugs* **2009**, *20* (2), 89−95. (b) Vukelja, S. J.; Anthony, S. P.; Arseneau, J. C.; Berman, B. S.; Cunningham, C. C.; Nemunaitis, J. J.; Samlowski, W. E.; Fowers, K. D. Phase 1 study of escalating-dose OncoGel (ReGel/paclitaxel) depot injection, a controlled-release formulation of paclitaxel, for local management of superficial solid tumor lesions. *Anti-Cancer Drugs* **2007**, *18* (3), 283−289.

(119) Jeong, B.; Bae, Y. H.; Kim, S. W. In situ gelation of PEG−PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. *J. Biomed. Mater. Res.* **2000**, *50* (2), 171−7.

(120) Rowinsky, E. K.; Eisenhauer, E. A.; Chaudhry, V.; Arbuck, S. G.; Donehower, R. C. Clinical toxicities encountered with paclitaxel (Taxol). *Semin. Oncol.* **1993**, *20* (4 Suppl 3), 1−15.

(121) (a) Schiff, P. B.; Fant, J.; Horwitz, S. B. Promotion of microtubule assembly in vitro by taxol. *Nature* **1979**, *277* (5698), 665−667. (b) Tishler, R. B.; Geard, C. R.; Hall, E. J.; Schiff, P. B. Taxol sensitizes human astrocytoma cells to radiation. *Cancer Res.* **1992**, *52* (12), 3495−3497. (c) Tishler, R. B.; Schiff, P. B.; Geard, C. R.; Hall, E. J. Taxol: a novel radiation sensitizer. *Int. J. Radiat. Oncol., Biol., Phys.* **1992**, *22* (3), 613−7. (d) Mekhail, T. M.; Markman, M. Paclitaxel in cancer therapy. *Expert Opin. Pharmacother.* **2002**, *3* (6), 755−766. (e) Nsereko, S.; Amiji, M. Localized delivery of paclitaxel in solid tumors from biodegradable chitin microparticle formulations. *Biomaterials* **2002**, *23* (13), 2723−31.

(122) Prasad, A.; Loh, X., From Bench to Bedside—An Example of an In Situ Hydrogel in In Vivo Applications. In *In-Situ Gelling Polymers*; Loh, X. J., Ed.; Springer: Singapore, 2015; pp 215−226.

(123) (a) Matthes, K.; Mino-Kenudson, M.; Sahani, D. V.; Holalkere, N.; Fowers, K. D.; Rathi, R.; Brugge, W. R. EUS-guided injection of paclitaxel (OncoGel) provides therapeutic drug concentrations in the porcine pancreas (with video). *Gastrointestinal endoscopy* **2007**, *65* (3), 448−53. (b) Linghu, E.; Matthes, K.; Mino-Kenudson, M.; Brugge, W. R. Feasibility of endoscopic ultrasound-guided OncoGel (ReGel/paclitaxel) injection into the pancreas in pigs. *Endoscopy* **2005**, *37* (11), 1140−2.

(124) Tyler, B. M.; Hdeib, A.; Caplan, J.; Legnani, F. G.; Fowers, K. D.; Brem, H.; Jallo, J.; Pradilla, G. Delayed onset of paresis in rats with experimental intramedullary spinal cord gliosarcoma following intratumoral administration of the paclitaxel delivery system OncoGel Laboratory investigation. *J. Neurosurg. Spine* **2012**, *16* (1), 93−101.

(125) Appel, E. A.; Rowland, M. J.; Loh, X. J.; Heywood, R. M.; Watts, C.; Scherman, O. A. Enhanced stability and activity of Temozolomide in primary glioblastoma multiforme cells with cucurbit[n]uril. *Chem. Commun. (Cambridge, U. K.)* **2012**, *48* (79), 9843−5.

(126) Vellimana, A. K.; Recinos, V. R.; Hwang, L.; Fowers, K. D.; Li, K. W.; Zhang, Y.; Okonma, S.; Eberhart, C. G.; Brem, H.; Tyler, B. M. Combination of paclitaxel thermal gel depot with Temozolomide and radiotherapy significantly prolongs survival in an experimental rodent glioma model. *J. Neuro-Oncol.* **2013**, *111* (3), 229−36.

(127) DeWitt, J. M.; Krishnamurthy, S.; Neoral, C.; Nosek, V.; Duvall, G. A.; Kaminski, M.; Wallner, G.; Litka, P.; Daugherty, C.; Fowers, K. 532 Multicenter, Prospective Randomized Phase IIB Study of Endoscopic Ultrasound Guided Intratumoral Injection of OncoGel (Regel/Paclitaxel) As a Component of Neoadjuvant Chemoradiotherapy for Local or Loco-Regional Operable Esophageal Cancer - Interim Safety Results. *Gastrointestinal Endosc.* **2011**, *73* (4 Supplement), AB136.

(128) Williams, R. Discontinued drugs in 2011: oncology drugs. *Expert Opin. Invest. Drugs* **2013**, *22* (1), 9−34.

(129) http://www.cancer.gov/about-cancer/treatment/types/targeted-therapies/targeted-therapies-fact-sheet.

(130) Torres, A. J.; Zhu, C.; Shuler, M. L.; Pannullo, S. Paclitaxel delivery to brain tumors from hydrogels: a computational study. *Biotechnology progress* **2011**, *27* (5), 1478−87.

(131) Zhu, W.; Masaki, T.; Bae, Y. H.; Rathi, R.; Cheung, A. K.; Kern, S. E. Development of a sustained-release system for perivascular delivery of dipyridamole. *J. Biomed. Mater. Res., Part B* **2006**, *77* (1), 135−43.

(132) Lei, K.; Shen, W.; Cao, L.; Yu, L.; Ding, J. An injectable thermogel with high radiopacity. *Chem. Commun.* **2015**, *51* (28), 6080−3.

(133) Choi, B.; Loh, X. J.; Tan, A.; Loh, C. K.; Ye, E.; Joo, M. K.; Jeong, B., Introduction to In Situ Forming Hydrogels for Biomedical Applications. In *In Situ Gelling Polymers*; Loh, X. J., Ed.; Springer Science: Singapore, 2015; pp 5−35.

DOI: 10.1021/acsbiomaterials.5b00515
ACS Biomater. Sci. Eng. 2016, 2, 295−316