

Thermogels: In-situ Gelling Biomaterial

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Keywords: injectable hydrogels, thermo-sensitive polymers, LCST, sol-gel transition.

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 entrapped therapeutic payload by mixing. Upon injection, the higher physiological temperature causes gelation *in-situ* due to the presence of thermo-sensitive 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 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 thermogels recently developed 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 focus on OncoGel, an experimental thermogel that demonstrated excellent individual or synergistic drug delivery system in pre-clinical 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.

Introduction

Hydrogel is an important class of soft material that is suitable for a wide range of biomedical applications owing to their high water content and tunable properties. These hydrophilic three dimensional polymeric networks formed by chemical or physical crosslinks can hold a large amount of water without disintegration. Chemically-crosslinked hydrogels are typically tough and elastic, creating highly demanded properties in dynamic environments such as skin, cartilage and cardio-related devices¹. In contrast, hydrogels based on physically crosslinked 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* experiments³.

Thermo-responsive 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 physiological temperature⁴. 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) high water content of the hydrogel 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) biodegradable thermogel can be excreted from body after achieving its intended purpose; vi) the rate of drug release can be easily tailored by changing the formulation^{4c}.

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 learnt from Oncogel development and other current cancer treatment formulations are also highlighted.

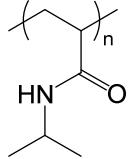
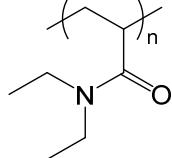
1. Synthesis and Self-assembly of *In-situ* Forming Gels

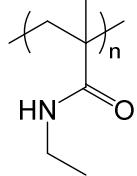
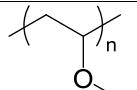
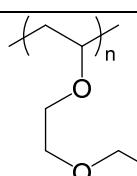
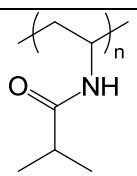
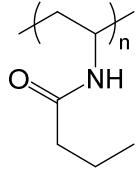
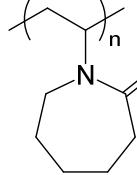
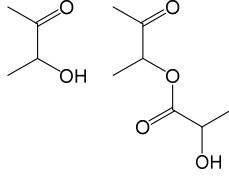
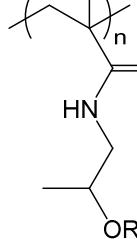
Thermogels exhibit reversible sol-gel transition as temperature changes. This phase change behavior is reversible as the gel is formed by physical crosslinks between the polymer chains. These thermoresponsive copolymers consist of hydrophilic and hydrophobic segments, which can self-assemble into polymeric 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 crosslinks 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 acryl amide) (PNIPAAm), poly(vinyl ether) (PVE), poly(N,N-diethylacrylamide) (PDEAM), poly(N-vinyl alkyl amide), and poly(N-vinyl caprolactam), as listed in Table 1⁵. Roy and co-workers listed a comprehensive table of various thermo-responsive polymers and their respective LCST and upper critical solution temperature (UCST)⁶. These polymer-water systems form a gel below UCST and become solution above it. Ward et al. discussed phase change behavior of these polymers⁷. There are few studies describing about the hydrogels that consisted of polymers with UCST behavior, for example gelatin (UCST about 30 °C), copolymers of poly(acrylamide) and poly(acrylic acid)⁸, poly(N,N-dimethyl(acrylamidopropyl) ammonium propane sulfonate)⁹, copolymers of poly(allyurea) and poly(L-citrulline)¹⁰, copolymers of poly(allyurea) and poly(allyamine)¹¹, poly(N-acryloyl glycaminamide)¹².

Table 1. LCST of several typical thermo-responsive polymers⁵

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)

1.1 PEG-based Block Copolymers – via Ring Opening Polymerization

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2 PEG-PPG-PEG triblock copolymers (ABA type), also known as Pluronic (BASF) or
3 Poloxamer (ICI) consist of 30 % PPG hydrophobic segment and 70 % PEG hydrophilic
4 segment. Long term drug release profile is not feasible as these gels erode within a few days
5 *in vivo*. Numerous studies have provided various alternative modifications, including
6 crosslinking¹³, grafting¹⁴, copolymerization¹⁵ or substituting PPG to other polyesters such as
7 PLGA¹⁶, PCL¹⁷, and poly([R]-3-hydroxybutyrate) (PHB)¹⁸ (Figure 1, 1-4). These ABA type
8 triblock copolymers are synthesized in a two-step reaction: ring opening polymerization of B
9 block using methoxy-PEG as initiator, followed by condensation reaction to link two B
10 blocks together using diisocyanate as coupling agent.
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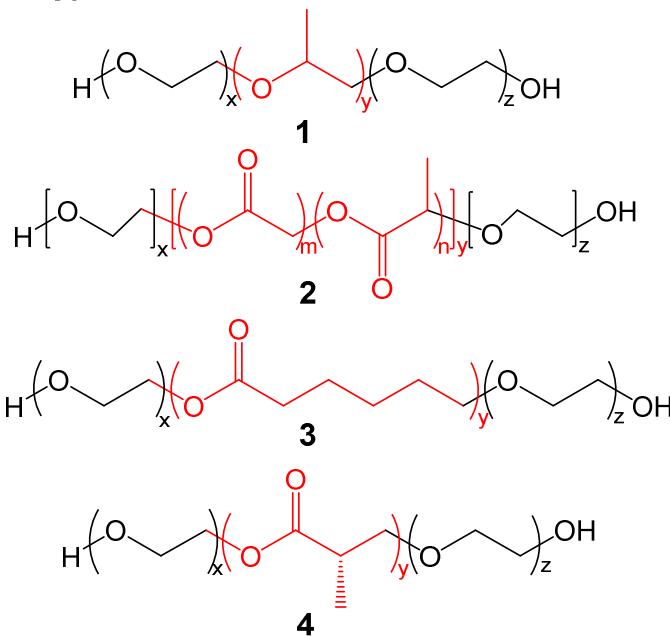
25 Substituting PPG block with more hydrophobic segment will affect the overall physical
26 nature of the polymer. Replacing PPG with PLGA significantly increased the sustainment of
27 gel duration for up to a few weeks. Increasing hydrophobicity enhances thermodynamic
28 interaction associated with gelation. As demonstrated by the increased of hydrophobic PLGA
29 chain length, the gelation temperature and gelation concentration decreased. By substituting
30 with PCL, which is more hydrophobic than PLGA, PEG-PCL-PEG forms a gel at a lower
31 polymer concentration as compared to PEG-PLGA-PEG. PLGA (G:L ratio 2:8) and PCL
32 exhibits three and ten times more hydrophobicity respectively than PPG^{17b}. Li et al. studied
33 PEG-PHB-PEG, in which PHB has typically higher hydrophobicity than most biodegradable
34 polyesters¹⁸. While the system can form micelles in aqueous environment, thermogelation is
35 not achievable at any temperature possibly due to imbalanced hydrophilic-hydrophobic ratio.
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52 BAB-type triblock thermogels, especially PLGA-PEG-PLGA, have been studied intensively
53 since 2000¹⁹. The synthesis of BAB type amphiphilic copolymers is easier as compared to the
54 ABA type. PLGA-PEG-PLGA (1500-1000-1500) thermogel (commercially available as
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2 ReGel[®]) is used in release studies of proteins and conventional drugs^{19c}. It is prepared by ring
3 opening polymerization of lactic acid and glycolic acid cyclic monomers, using PEG 1000 Da
4 as the initiator and tin octoate as the catalyst. Sol-gel transition of PLGA-PEG-PLGA (BAB
5 type) occurs at a lower temperature due to enhanced hydrophobic interaction which accounts
6 from more hydrophobic segments in the system compared to PEG-PLGA-PEG (ABA type).
7 Furthermore, modifications on the end groups from hydrophilic hydroxyl terminals to
8 hydrophobic alkyl chains (CH_2CH_3) significantly decreases the sol-gel transition temperature
9 by 10 °C^{19a, 20}. The gelation concentration also decreases from 12 wt.% to 2 wt.% (Figure 1,
10 **5a** and **5b**). The research group also reported end group effect on thermogelling properties
11 with ionizable-end group, affecting hydrophilic/hydrophobic balance²¹. These studies draw a
12 clear conclusion: tuning the balance of hydrophilicity and hydrophobicity is the key to
13 achieve thermogelation.

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16 Physical gelation of PLGA-PEG-PLGA thermogel in water was studied using TEM, ¹³C
17 NMR and DLS^{19a}, the physical observation and schematic drawing of micelles network were
18 shown in Figure 2. Gelling mechanism was affected by ordered-packing of micelles. At sol
19 state, micelles were formed by self-assembly of amphiphilic block copolymers, with
20 hydrophobic PLGA and hydrophilic PEG form the core and the corona respectively. As
21 temperature increases to the sol-gel transition temperature, the micelles aggregate into
22 percolated micellar network via hydrophobic interactions²². The gel turns opaque as the
23 micelle aggregates grow into a network with a mesh size of visible light wavelength. As
24 temperature continues to rise, excessive hydrophobicity destroys the micellar structure which
25 leads to macroscopic precipitation.

ABA type



BAB type

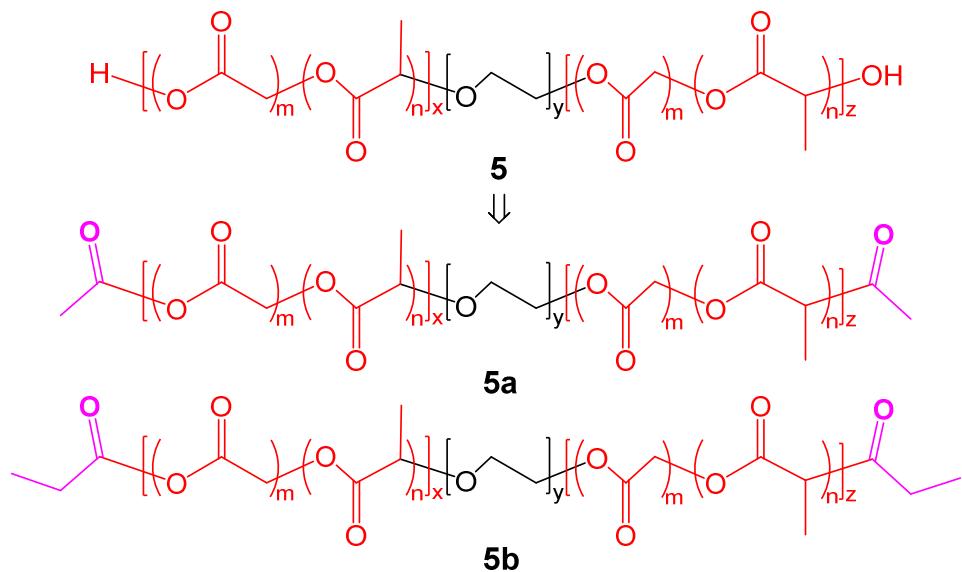


Figure 1. Chemical structure of ABA type and BAB type triblock copolymers. (red: hydrophobic segments, pink: additional hydrophobic ends, blue: pH-responsive segments) **1:** Pluronic PEG-PPG-PEG **2:** PEG-PLGA-PEG **3:** PEG-PCL-PEG **4:** PEG-PHB-PEG **5:** PLGA-PEG-PLGA **5a:** diacetate PLGA-PEG-PLGA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

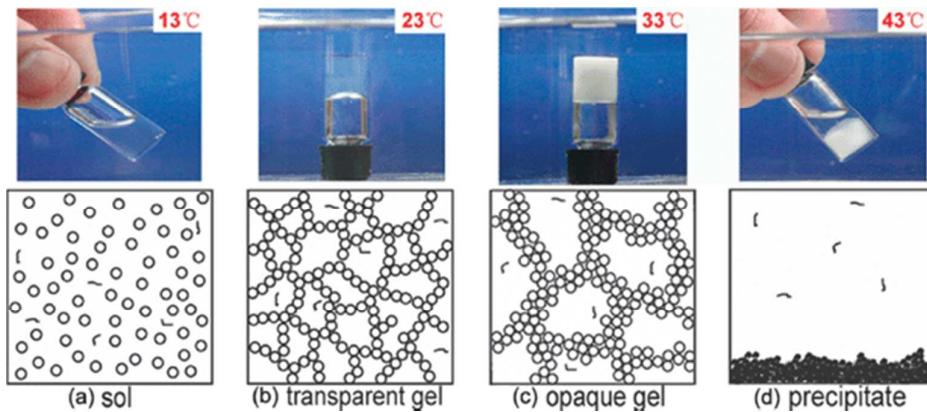


Figure 2. Visual observation (above) and schematic drawing of PLGA-PEG-PLGA micellar network showing thermogelling behavior (below). Reproduced from Yu et al.^{19b}

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 blocks²³. Addition of salts (e.g. NaCl) can significantly tune the sol-gel transition temperature and critical gelation concentration of triblock copolymers¹⁶. Mixing of two non-thermogellable copolymers can sometimes lead to thermogellation²⁴.

Recently, studies on PF127 conjugation revealed possible tunable properties. Shachaf et al. reported the synthesis of PF127 and fibrinogen crosslinked hydrogel, prepared by photopolymerization of acrylated PF127^{13a}. Another study reported PF127 double-crosslinked network prepared via physical mixing of PF127 gels and carboxymethyl chitosan in the presence of glutaraldehyde. At physiological temperature, the glutaraldehyde-carboxymethyl chitosan crosslinks formed interpenetrate the PF127 gel^{13b}. Moreno et al. demonstrated that mechanical and bio-adhesive properties of PF127 gel significantly

improved after conjugation with poly(methyl vinyl ether-co-maleic anhydride) (Gantrez[®]) via ring opening polymerization¹⁵.

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 thermo-sensitive polymers prepared by ring opening polymerization of N-carboxy anhydride using methoxy-PEG (mPEG) as initiator. Transition temperatures between 25 °C 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 water²⁵. 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, while lower transition temperatures could be achieved by using more hydrophobic oligo-peptides. Various applications in drug delivery²⁵ and wound healing²⁶ 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 multi-block 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

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2 increases²⁷. Sol-gel transition of a polyurethane aqueous solution is achieved by self-
3 assembly of hydrophilic and hydrophobic segments into micellar structure at elevated
4 temperature.
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8 Incorporation of a small amount (1-5%) of third component (a hydrophobic diol) into the
9 multiblock PEG/PPG polyurethane could tune the properties of thermogelling copolymers. A
10 study has shown that PHB added could lower the critical gelation concentration (CGC) of the
11 aqueous system. Poly(ϵ -caprolactone) (PCL)²⁸, poly(trimethylene carbonate) (PTMC)²⁹ or
12 PLA³⁰ imparts biodegradability, while poly(ethylene butylene)³¹ provides bio-stability. The
13 sol-gel transition mechanism of poly(PEG/PPG/PHB urethane)s was reported in³², as shown
14 in Figure 3. The long polymers chains of PEG, PPG and PHB are connected by urethane
15 linkages. Associated micelles are formed by the amphiphilic multiblock copolymers at a
16 highly diluted solution (99.9% water, 0.1% polymer). These self-assembled micelles have
17 PEG hydrophilic tails that interact with water and hydrophobic cores that consist of PPG and
18 PHB. A minimum polymer concentration (2-5 wt.%) and optimum micelle concentration, is
19 necessary for gel formation. At low temperature, the aqueous solution is clear because the
20 PPG segments behave more hydrophilic causing the polymers to be well-solvated in water.
21 Increase in temperature resulted in the dehydration of PEG segments, and PPG segments
22 behave more hydrophobic above its LCST, becoming less water-soluble. When
23 hydrophobic/hydrophilic balance in the system is achieved, a gel state is reached. Micellar
24 aggregation due to self-association of PEG corona and increased hydrophobicity of PPG
25 drives the gel formation. Increase in temperature resulted in severe dehydration and collapse
26 of the PEG corona, exposing the hydrophobic core to form a turbid solution. PHB added as a
27 third diol component in this system enhanced the hydrophobicity, leading to a lower CGC as
28 compared to poly(PEG/PPG urethane).
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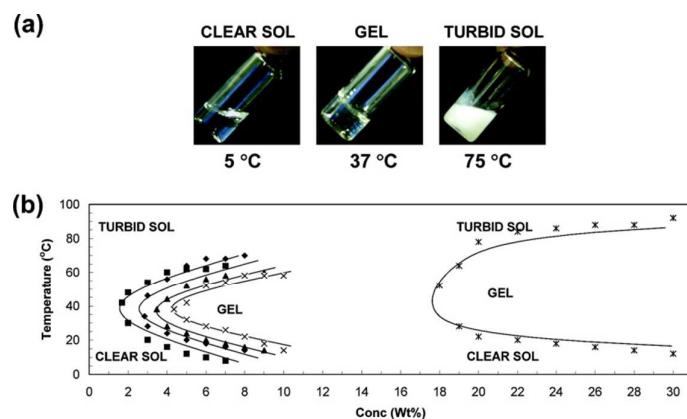


Figure 3. (a) Sol-gel transition of poly(PEG/PPG/PHB urethane)s (b) Phase-diagram of the multiblock polyurethane as compared to PF127. Reproduced from Loh et al.³³

1.4 Poly(N-isopropyl acryl amide)-based block copolymers – via ATRP and RAFT Polymerization

Poly(N-isopropyl acryl amide) (PNIPAAm) pioneered the development of reversible thermo-sensitive hydrogel. Since 1960s, numerous studies on synthesis of thermo-responsive PNIPAAm and its derivatives have been reported for biomedical applications such as drug delivery, cell encapsulation and cell culture sheets³⁴. PNIPAAm known for having low LCST at 32 °C in aqueous solution is relatively insensitive to the changes in pH, concentration or chemical environment³⁵. 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 copolymers³⁶. 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. Thermo-responsive PNIPAAm-[hydrophobic core]-PNIPAAm and PNIPAAm-[hydrophilic core]-PNIPAAm copolymers can be obtained by ATRP³⁷. 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 inter-micellar bridging. Recently, Li et al. prepared lignin-b-PNIPAAm-b-(PEG-co-PPG) copolymers consisting of lignin as the hydrophobic core, PEG as the hydrophilic corona, and PPG and PNIPAAm as thermo-sensitive segments. These thermo-sensitive segments transform from hydrophilic to hydrophobic as the temperature increases³⁸. 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.

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2
3 RAFT polymerization is another method employed to obtain multiblock amphiphilic
4 copolymers³⁹. As compared to ATRP multi-step alternating addition of two types of
5 monomers into a living polymerization system, RAFT polymerization is relatively easier to
6 prepare with narrow polydispersity index. Using cyclic- or polytrithiocarbonates as the chain
7 transfer agent, a multiblock PNIPAAm-PDMA copolymer was prepared with a two-step
8 addition, as shown in Figure 4. The chain length of each sequence can be tuned by the ratio of
9 monomer and trithiocarbonates⁴⁰. Recent study highlighted the feasibility to prepare low
10 LCST blocks of PNIPAAm and poly(N,N-diethylacrylamide) (PDEA) in an aqueous
11 environment at 25 °C⁴¹. RAFT polymerization requires no metal-ligand complex as catalyst
12 for the polymerization; complex purification procedure can thus be avoided. Double
13 hydrophilic block copolymers (DHBC) have been synthesized using PNIPAAm and
14 poly(N,N-dimethylacrylamide) (PDMA) via consecutive RAFT polymerization technique^{40,}
15 ⁴². The copolymers showed thermally-induced unimolecular or multimolecular micelles
16 aggregation based on different copolymer architecture, as shown in Figure 5. Physical gels
17 are formed when the multi-block PNIPAAm-PDMA consisted of PNIPAAm and PDMA with
18 certain sequence length, due to the formation and aggregation of unimolecular micelles. In
19 contrast, no gel formation is observed when multimolecular micelles aggregate. Addition of
20 salts can significantly reduce critical gelation concentration and critical gelling temperature,
21 by establishing a correlation among the Hofmeister effect, aggregation behavior and gelation
22 properties⁴².

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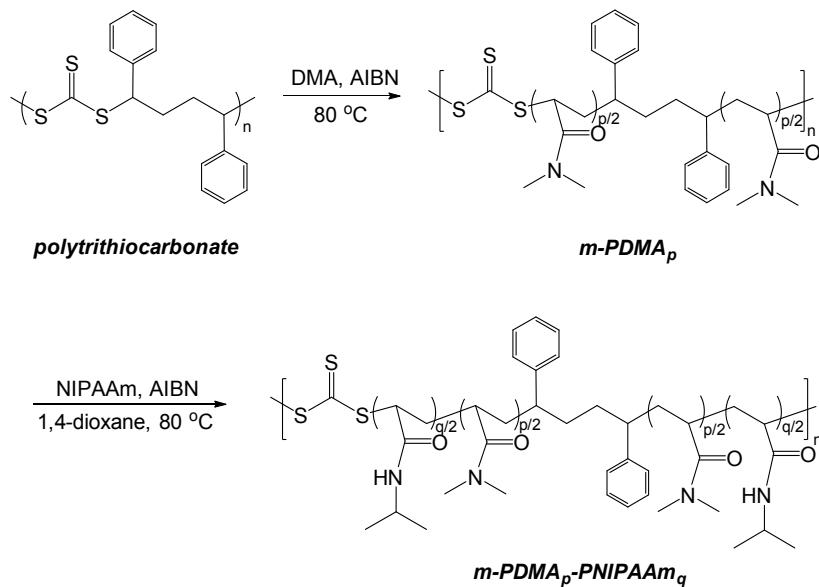


Figure 4. Synthesis of multiblock copolymers m-PDMA-PNIPAAm by successive RAFT polymerization, using polytrithiocarbonate as chain transfer agent. Reproduced from Ge et al.⁴²

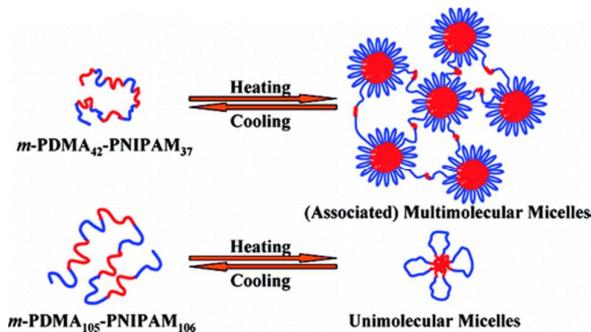


Figure 5. Schematic drawing of multiblock PDMA-PNIPAAm showing thermally-induced unimolecular or multimolecular micelles aggregation (Red=PNIPAAm, Blue=PDMA). Reproduced from Zhou et al.⁴⁰

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 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 PEG⁴³. In addition to its thermosensitivity, PoEGMA shows protein-repellant properties which are of great interest as non-fouling surface applications⁴⁴. Influence of molecular structure on the thermoresponsive properties of PoEGMA and PoEGA as biomaterials have been discussed⁴⁵. As shown in Figure 6, 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 techniques⁴⁶. PoEGMA showed a range of LCST (P2 to P9, in Figure 5) from 26–90 °C depending on the number of PEG side chain repeating units⁴⁷.

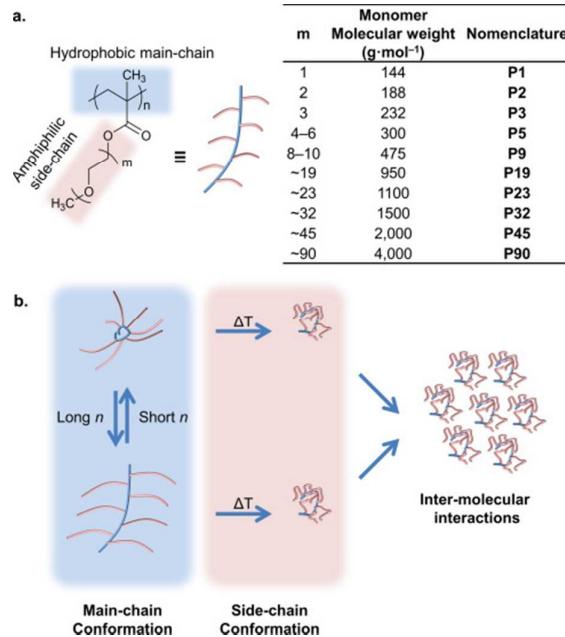


Figure 6. (a) Chemical structure of PoEGMA (main and side chains) with different number of repeating units, nomenclature, and their molecular weights. (b) Chain length effect: main

chain and side chain conformation, and molecular self-assembly at elevated temperature.

Reproduced from Liu et al.^{45a}

2. Evaluating the Biodegradability of Thermogels

Biodegradability of thermo-sensitive 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, ¹H 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 studied⁴⁸. However, limitation of these polyethers-based thermogelling copolymers lies on the non-biodegradable 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 unfavourable 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

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2 degradable blocks^{4c, 16, 17b, 49}. In addition, polypeptide-based thermogelling systems
3 containing enzymatically degradable peptides such as poly(alanine-co-phenyl alanine) (PAF)
4 and poly (L-alanine) (PAL) were used in the synthesis of thermoresponsive copolymers.
5 These systems demonstrated good *in vitro* stability in aqueous solution and *in vivo*
6 degradability at the presence of proteolytic enzymes⁵⁰.
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16 2.2 Rate of Degradation 17

18 The core motivation behind controlled drug delivery is achieving tunable rate of degradation
19 *in vitro* and *in vivo*. Rate of degradation can be tailored by 1) block length, 2) end group
20 modification, 3) type of biodegradable block, 4) composition of the copolymers, 5)
21 environmental effect. Tailoring these components could affect drug release rate and drug
22 delivery profile. Some studies revealed that different physiological environment (presence of
23 enzymes, ionic exchange and constant flow of body fluid) *in vitro* and *in vivo* could affect
24 degradation profiles. This sub-section will discuss research on degradation of thermogels in
25 both *in vitro* and *in vivo*.
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38 *In vitro* degradation rate of thermogelling PEG-PLGA-PEG copolymers is affected by the
39 block length of hydrophobic component (2310 and 2810 Da) and polymer concentration in
40 water (20, 27 and 33 wt.%). Thermogels with longer hydrophobic block (*i.e.* PLGA) or
41 higher polymer concentration in water, resulted in slower degradation⁵¹. *In vivo* degradation
42 demonstrated rapid sol-gel transition (33 wt. % of polymer in aqueous solution) upon
43 subcutaneous injection in the rat with stable three dimensional gel shape maintained for more
44 than 1 month⁵².
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3 Kim et al. reported on sulfamethazine oligomers (SMOs) added to both ends of thermo-
4 sensitive PCLA–PEG–PCLA block copolymer to impart pH- and thermo-sensitivity⁵³. *In*
5 *vitro* evaluation on block copolymer-SMO gel showed slower degradation rate due to
6 buffering effect of sulfonamide groups. The presence of sulfonamide groups neutralized the
7 accelerated degradation effect as a result of accumulation of degradation products e.g. lactic
8 acid and caproic acid from PCLA segment. *In vivo* degradation of the block copolymer-SMO
9 gel showed complete degradation within 6 weeks.
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20 *In vitro* degradation studies of thermogelling poly(ester urethane)s based on PEG and PPG
21 were reported. PHB, PLLA or PCL are added on these PEG/PPG based poly(ester urethane)s
22 to impart biodegradability^{28, 49b, 54}. Different rates of degradation can be achieved by
23 changing the degradable polyester groups and composition in the thermogelling copolymers.
24 Copolymer gel (5 wt.% polymer in aqueous solution) consisted of PHB segment could be
25 completely eroded in 30, 40, and 70 days, inversely proportional with PHB content of 8, 5,
26 and 2 wt.%, respectively. Structural deterioration was observed at day 14, as visualised by
27 SEM analysis (Figure 7). However, the molecular weight of the copolymer (M_n) remained
28 half of the initial M_n (~60 000 Da) after six months of incubation⁵⁴. These observations
29 suggested that the copolymer gel consisted of PHB follows a typical surface degradation
30 model. Typical surface degradation of polyesters proceeds with mass loss at constant
31 velocity, with molecular weight decrement observed at the later stage⁵⁵, which is similar with
32 the poly(PEG/PPG/PHB) thermogel. Another poly(ester urethane) copolymer containing
33 PLLA segment was synthesized using the same method³⁰. The copolymer gel degraded at a
34 much faster rate than its PHB counterpart. The gel (10 wt.% polymer in aqueous solution)
35 hydrolytically degraded to polymer fragments with M_n lower than 10000 Da (initial M_n)
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3 25800 Da) within three months (Figure 8). These fragments can potentially be excreted from
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5 the body via renal filtration.
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Different types of biodegradable hydrophobic blocks were studied. Thermo-sensitive diblock mPEG–copolymers 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 initiator⁵⁶. *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.

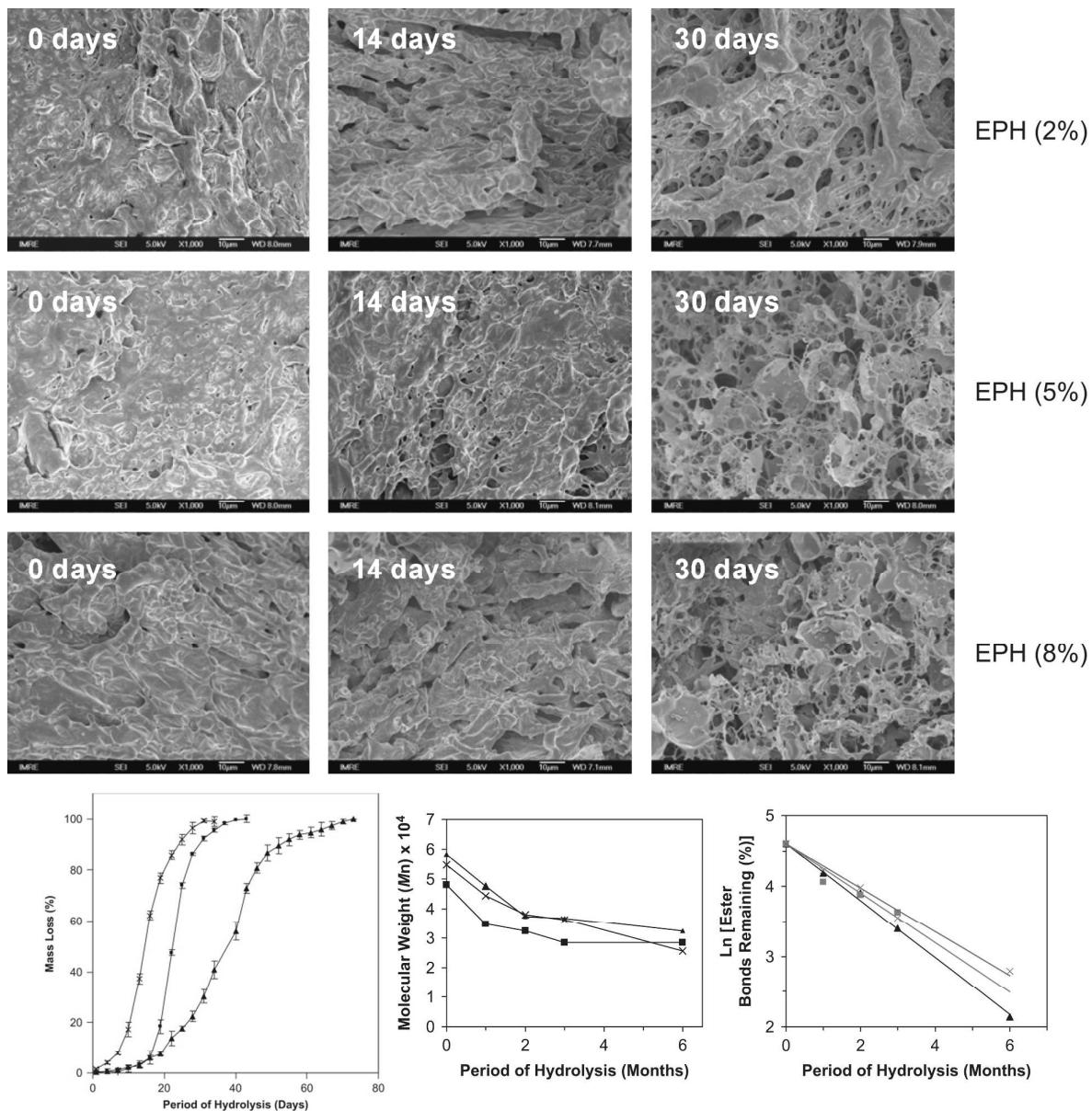


Figure 7. SEM images of hydrogel residues after various periods of degradation in PBS at pH 7.4 and 37 °C. Bottom: (left) Mass loss (%) of the poly(PEG/PPG/PHB urethane) hydrogels (5 wt. %) after incubation in PBS at pH 7.4 and 37 °C. (middle) Changes in molecular weight of the copolymer degradation 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 (\blacktriangle : EPH(2%), \blacksquare : EPH(5%), \times : EPH(8%)). Reproduced from Loh et al.⁵⁷

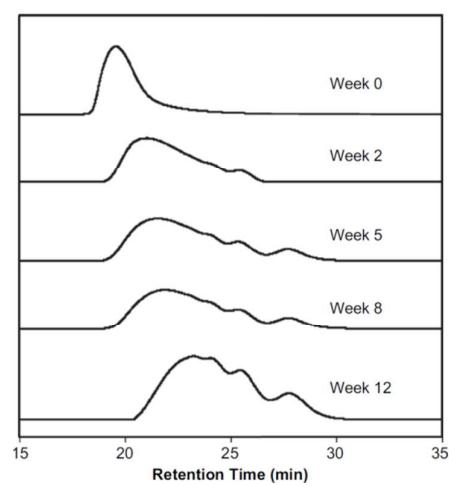


Figure 8. GPC profile of remaining poly(PEG/PPG/PLA urethane)s gels incubated in a porous cellulose cassette at various degradation intervals at pH 7.4. Reproduced from Loh et al.^{49b}

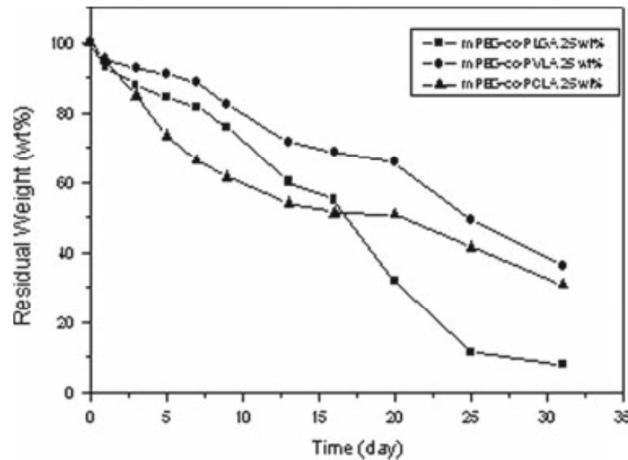


Figure 9. Degradation behavior of mPEG–polyester diblock copolymers determined using the weight loss method. (● : mPEG-*b*-PVLA, ▲ : mPEG-*b*-PCLA, ■: mPEG-*b*-PLGA) Degradation curve of mPEG-*b*-PPLA was not shown in figure. Reproduced from Chen et al.⁵⁶

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 thermogel⁵⁸. 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 disulphide bonds *in vivo*. In another study, rate of degradation was tuned by adding hydroxyapatite (HA) into the thermogels⁵⁹. *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). 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 four weeks post-implantation, attributed to the acidic degradation products originated from PLGA segments. After eight weeks, the absence of inflammatory cells, increased presence of fibroblasts and creation of new blood vessels, suggested biocompatibility of the thermogel composite.

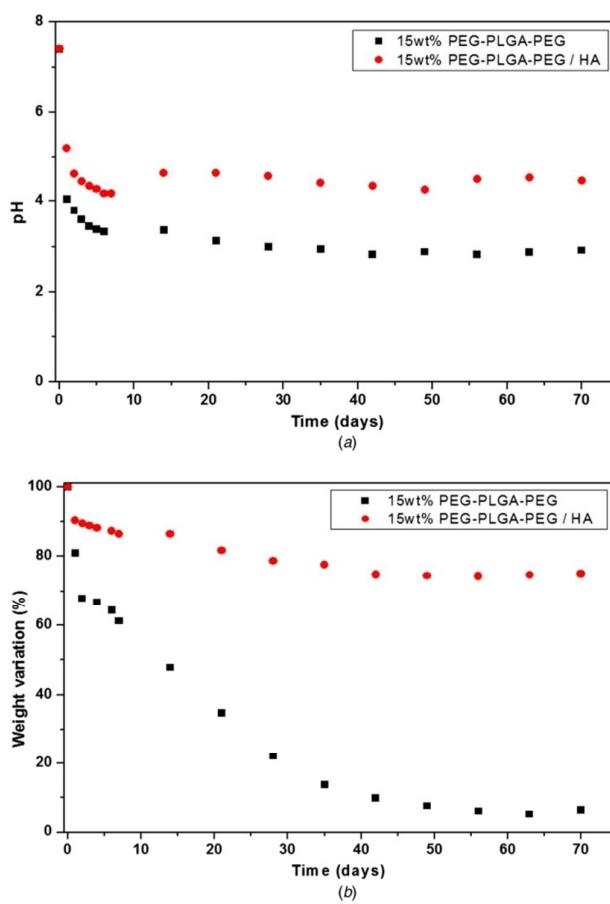


Figure 10. *In vitro* degradation of PEG-PLGA-PEG hydrogel and PEG-PLGA-PEG/HA hydrogel composite: (a) pH variation; and, (b) weight variation during incubation in phosphate buffer (pH = 7.0) at 37 °C. Reproduced from Lai et al.⁵⁹

Studies have been reported on a group of polypeptide-based thermogels that are stable in water, but degradable *in vivo* by 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 rats²⁵. The gel lost more than 90 % of its original mass in 15 days of *in vivo* incubation, while 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* incubation⁶⁰. PEG-*b*-

PAF which degrades faster than PAL-PLX-PAL showed marginal tissue inflammation, while 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 sub-section, a variety of biodegradable thermo-sensitive 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 velocity⁵⁵; bulk erosion of polyesters changes the rate of erosion with time⁶¹, as depicted in Figure 11. Most 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 dimensions⁶². For example, PHB degrades via surface erosion due to its inherent high hydrophobicity that limits the diffusivity of water in the matrix; while 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 crosslinked thermogels undergo chain scission of the crosslinks before polymer erosion

occurs⁶³; while physically crosslinked 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 crosslinks and fast dissolution of hydrogel; 3) slow mass loss towards the end of the erosion.

Physiological environment of the biological entity also affected the nature of degradation mechanism. Polypeptide-based thermogels are non-degradable *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 elastase⁶⁰, while PEG-*b*-PAF-based thermogels showed degradation at the presence of cathepsin C, cathepsin B, elastase, chymotrypsin, and collagenase²⁵.

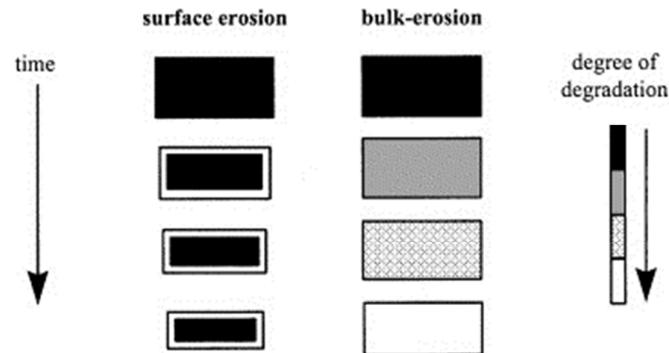


Figure 11. Schematic illustration of the changes a polymer matrix undergoes during surface erosion and bulk erosion. Reproduced from Burkersrod et al.^{62b}

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

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3 chemical entities post-degradation, 2) the property of degradation products may affect the
4 environment after being released from the gel matrix, 3) degradation debris might undergo
5 phagocytosis or pinocytosis. Therefore, extravasation process should be carefully monitored.
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12 **3. Applications in Drug Release**

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14 Thermogelling copolymers undergo sol-gel transitions as temperatures changes. Thus, while
15 such thermogels are injectable solutions with low viscosities at lower temperatures, they have
16 the ability to turn into gels upon injection into the physiological environment. Biodegradable
17 thermogels show extra benefits in their use as drug depots and delivery systems as they
18 require no follow-up surgical removal after depletion.
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28 **3.1 Drug Loading**

29 Hydrophobic drugs can be incorporated using the solvent-induced phase inversion technique
30 (SPI). A water insoluble polymer is first dissolved in an organic, water-miscible solvent
31 containing the drug. Upon its injection into the body and exposure to an aqueous
32 environment, the organic solvent dissipates out while water ingresses via diffusion⁶⁴. This
33 solvent exchange results in sol-to-gel transformation and polymer precipitation, leading to
34 implant formation (Figure. 12)⁶⁵.
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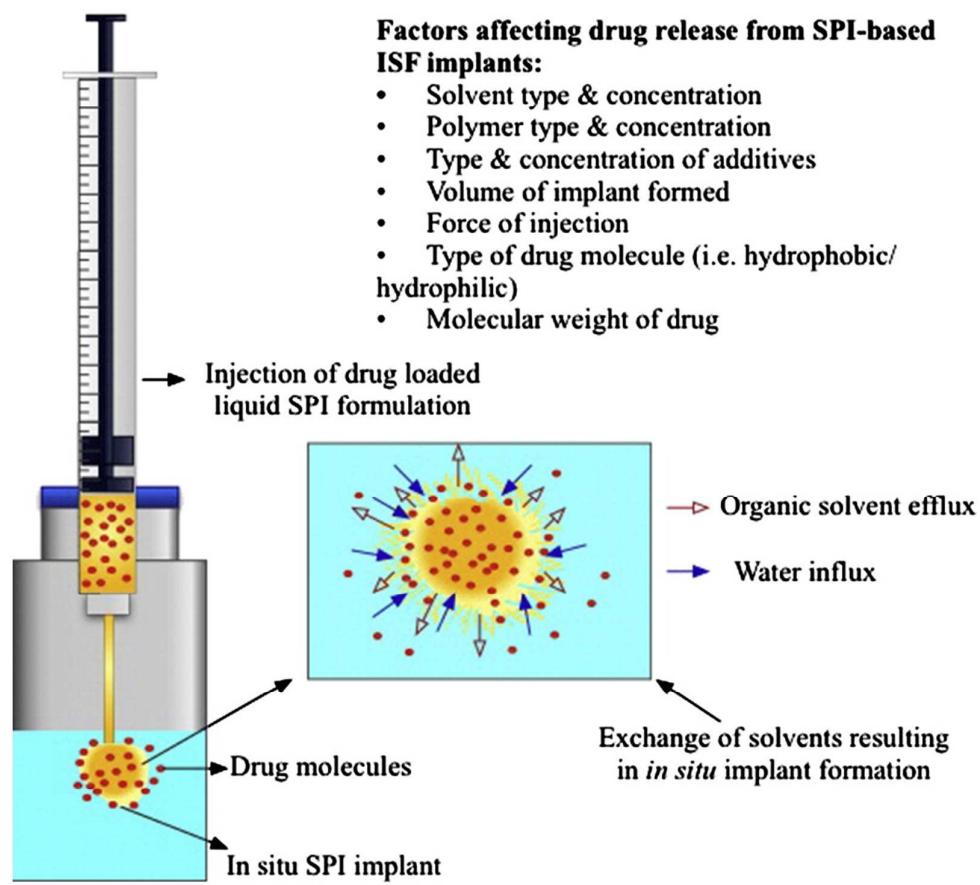


Figure 12. Schematic representations of solvent induced phase inversion technique (SPI) implant formation, solvent exchange and drug delivery. Reproduced from Thakur et al.⁶⁵

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.

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2 Pluronics gels are non-degradable. Furthermore, as these gels erode within a few days *in vivo*,
3 long term drug release is not feasible. As a result, a pluronic analog-based thermosetting gel
4 for ophthalmic drug delivery was developed⁶⁷. Pluronic analogs were incorporated with
5 mucoadhesive polysaccharide, sodium hyaluronate (HA-Na) for ocular retention. The
6 inclusion of F68 (10%) to F127 (21%) increased the phase transition temperature by 9 °C.
7 The formulation was a free flowing liquid below 25 °C and converted to a firm gel under
8 physiological conditions. Gamma scintigraphic data demonstrated that the precorneal
9 clearance of the thermosetting gel was significantly delayed as compared to the control
10 solution. This means that drug release can be prolonged through the use of these gels.
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13 The sustained release profile of protein from poly(PEG/PPG/PHB urethane) thermogels can
14 be controlled by tuning the concentration of thermogelling copolymers in the solution, where
15 a higher concentration of the copolymer leads to extended drug release period⁵⁴. A PEG–
16 PLGA–PEG (550–2310–550 and 550–2810–550) amphiphilic copolymer for drug release
17 were reported⁵¹. Figure 13(a) showed the release profile of a hydrophobic drug
18 (spironolactone) from the reported copolymer. Similar to poly(PEG/PPG/PHB urethane)
19 thermogels, it was observed that a higher copolymer concentration leads to a slower drug
20 release rate. This is because the higher initial polymer concentration resulted in a tighter
21 close-packed structure of the gel and thus reduced pore size and permeability of the drug.
22 However, highly concentrated polymer solutions should be avoided for drug delivery due to
23 changes in osmolality, transparency, and kinetics of gelation of solution. Apart from initial
24 polymer concentrations, the release rates can also be controlled by increasing the length of
25 the hydrophobic blocks, causing a slower release rate at the degradation dominant stage due
26 to an increased hydrophobicity in the gel. The release profile of a hydrophilic drug
27 (ketoprofen) from the aforementioned reported copolymer was also studied by the same
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research group, leading to the conclusion that the release rate of hydrophilic drugs was diffusion-controlled.

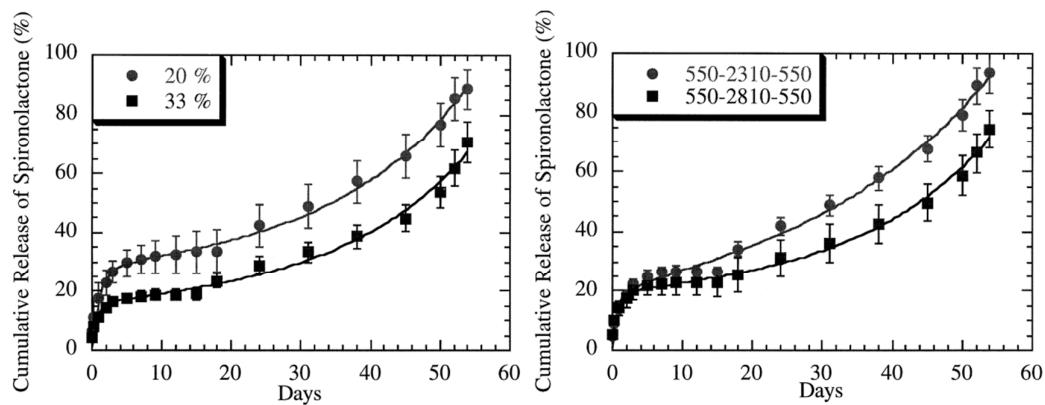


Figure 13. The release of hydrophobic drug (spironolactone) reflects the degradation rate of the polymers. Left: Higher gel hydration leads to a faster degradation rate of PEG–PLGA–PEG triblock copolymer. Right: Longer hydrophobic blocks in PEG–PLGA–PEG triblock polymers leads to smaller gel water content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

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–PLGA⁶⁸ or PEG–PPG–PEG⁶⁹ 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 anti-tumor drug was also confirmed by *in vivo* anti-tumor 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.

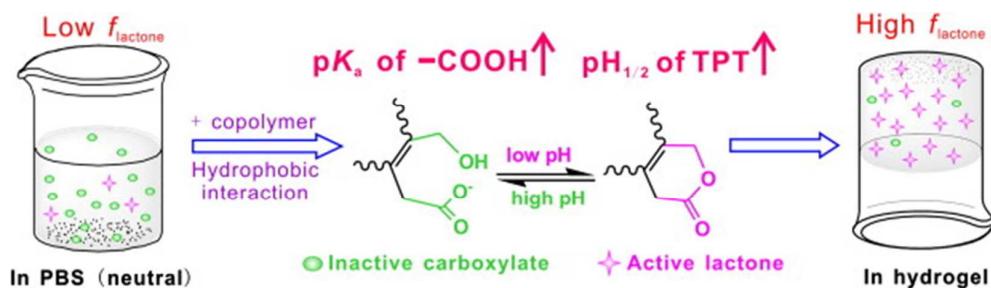


Figure 14. Schematic showing a thermoreversible hydrogel composed of copolymer PLGA–PEG–PLGA, which was found to enhance the equilibrium fraction of the lactone form of topotecan via elevation of the carboxylate pKa due to drug–material interactions. Reproduced from Chang et al.^{68b}

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 anti-tumor drugs, bovine serum albumin and glucoregulatory polypeptide- exenatide have also been delivered with PLGA–PEG–PLGA⁷⁰. Moreover, some water-soluble drugs, such as DOX⁷¹, can also 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) 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 release⁷³. The GLP-1 released from ReGel formulation *in vitro* and *in vivo* showed constant release for two 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 method⁶⁰. The thermogelation takes place in the syringe for 2 minutes at 37 °C before injection. Recently, an injectable *in situ*-forming gel named PME consisting of phospholipids, medium chain triglycerides (MCTs), and ethanol was developed⁷⁴. 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 anti-proliferative 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.

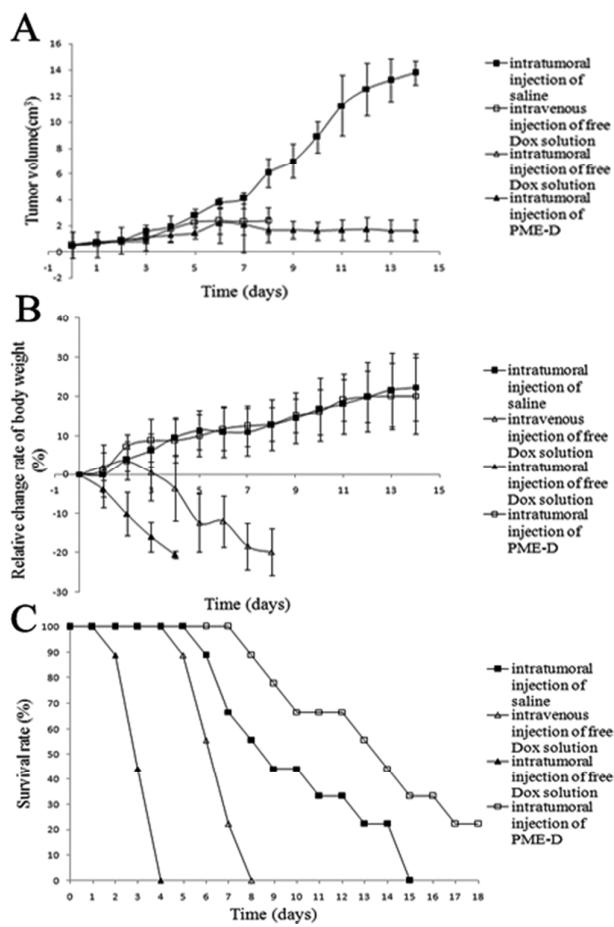


Figure 15. *In vivo* antitumor activity in mice bearing S180 sarcoma cancer cell xenografts.

The changes of tumor volume (A), relative body weight (B), and survival rate (C) ($p < 0.001$) were monitored to evaluate antitumor activity. Data is represented as the mean \pm standard deviation (SD) ($n = 9$). Reproduced from Wu et al.⁷⁴

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2 **4. *In-situ* Themogels for Tissue Engineering and Other Bio-applications**

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5 Tissue engineering (TE) and regenerative medicine are rapidly developing interdisciplinary
6 fields. Their aim is to develop biofunctional substitutes to replace or restore damaged tissues
7 caused by chronic disease or acute trauma. Tissue engineering involves three basic
8 components: cells, scaffolds and biomolecules. The major challenge for TE is to develop a
9 suitable scaffold that mimics the structure and biofunctions of the native extracellular matrix
10 (ECM). This scaffold should provide mechanical, spatial and biological signals for
11 regulating and guiding cell growth and tissue regeneration. Hydrogels with many advanced
12 properties such as viscoelastic nature, high water content mimicking ECM (70~80 %), and
13 amenability to chemical and physical modification, are highly attractive for biomedical
14 scaffold design⁷⁵. Unlike traditional 2D polystyrene culture plates, hydrogels can provide 3D
15 living environments for cells, resulting in different morphologies and cell expression of genes
16 and proteins. The injectability of minimally invasive *in-situ* gelling systems is an essential
17 consideration in the rational design of TE scaffolds. These gels allow direct injection to a
18 specific location and are able to conform to any desired shape. Grafted cells together with
19 functional bio-ingredients can be easily suspended in the *in-situ* gelling polymer aqueous
20 solution prior to injection. The hydrogels provide an aqueous 3D network matrix allowing for
21 cell attachment, proliferation, migration and even differentiation. By adjusting polymer
22 structure, molecular weight or concentration, the gel stiffness can also be tuned to favour
23 mechano-transduction-mediated tissue remodelling and regeneration. However, ethanol may
24 cause denaturation of some drugs such as proteins and peptides; thus, this system is not
25 suitable for the delivery of these drugs.

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29 **4.1 Cardiac Tissue Engineering**

As adult cardiomyocytes lack regeneration capacity, heart failure is currently incurable and leads to high morbidity and mortality worldwide⁷⁶. 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 thermo-sensitive hydrogels containing dextran chain grafted with PCL-(2-hydroxylethyl methacrylate) (HEMA) and PNIPAAm into infarcted myocardium to replace damaged ECM in rabbits⁷⁷. 30 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 thermo-responsive hydrogel based on copolymerization of N-isopropylacrylamide (NIPAAm), acrylic acid (AAc) and PTMC-HEMA for the treatment of chronic infarcted myocardium⁷⁸. 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 thermo-sensitive hydrogels based on PCL, NIPAAm, HEMA and dimethyl- γ -butyrolactone acrylate have also been synthesized by atom transfer radical polymerization⁷⁹. 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 (5kPa, 31kPa, 65kPa), 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-acryloylsuccinimide, 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

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3 showed the highest differentiation efficiency with developed calcium channels and gap
4 junctions for cell-cell interactions.
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10 Incorporation of functional bioingredients into hydrogels has been demonstrated to be an
11 effective approach in cardiac cell regeneration. A thermo-responsive amphiphilic hydrogel
12 was synthesized for the delivery of vascular endothelial growth factor (VEGF) plasmid into
13 hearts damaged by MI⁸¹. Up to 4 folds change in gene expression was observed using
14 hydrogel-based gene transfer as compared with the naked plasmid method. The injection of
15 VEGF-loaded plasmid hydrogels enhanced and sustained VEGF expression, and further
16 increased capillary density and larger vessel formation in the infarcted area. To provide
17 localized and sustained VEGF function and investigate its effects on cardiac recovery, a
18 temperature-sensitive, aliphatic polyester hydrogel conjugated with VEGF was prepared⁸².
19 After 35 days of implantation, the VEGF/hydrogel group enhanced blood vessel density,
20 attenuated adverse cardiac remodelling and improved ventricular function in a rat MI model.
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37 Basic fibroblast growth factor (bFGF) has also been incorporated into PNIPAAm-based
38 hydrogels for cardiac tissue engineering. These bFGF/hydrogel systems improved
39 angiogenesis and enhanced the cardiac differentiation of MSCs under ischemic conditions⁸³.
40 A novel injectable thermo-sensitive hydrogel consisting of a copolymer with N-
41 isopropylacrylamide/acrylic acid/2-hydroxylethyl methacrylate-poly(epsilon-caprolactone)
42 bioconjugated with type I collagen enhanced the survival of the grafted MSCs in the
43 myocardium. This led to enhanced neovascularization, decreased interstitial fibrosis, and thus
44 enhanced heart function⁸⁴. A thermo-sensitive chitosan chloride-glutathione (CSCl-GSH)
45 hydrogel was prepared by conjugating glutathione on chitosan chloride⁸⁵. These CSCl-GSH
46 hydrogels not only showed excellent biocompatibility to support cardiomyocyte adhesion and
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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 hydrogel which are electrically conductive supports electrical stimulation of cell-tissue constructs and regulates the growth of cardiac-myocytes^{76b, 76d}. 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. Multi-walled CNTs (MWCNTs) were interpenetrated into PNIPAAm hydrogel to prepare cell sheets for cardiac tissue engineering³⁴. 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 hydrogel⁸⁶. 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 18). Highly biocompatible tetraaniline (TA) is another electroactive material which has been incorporated into thermo-sensitive PNIPAAm-based copolymers⁸⁷. These electroactive and thermo-sensitive hydrogels were found to promote the proliferation and intracellular calcium transients of H9c2 cells.

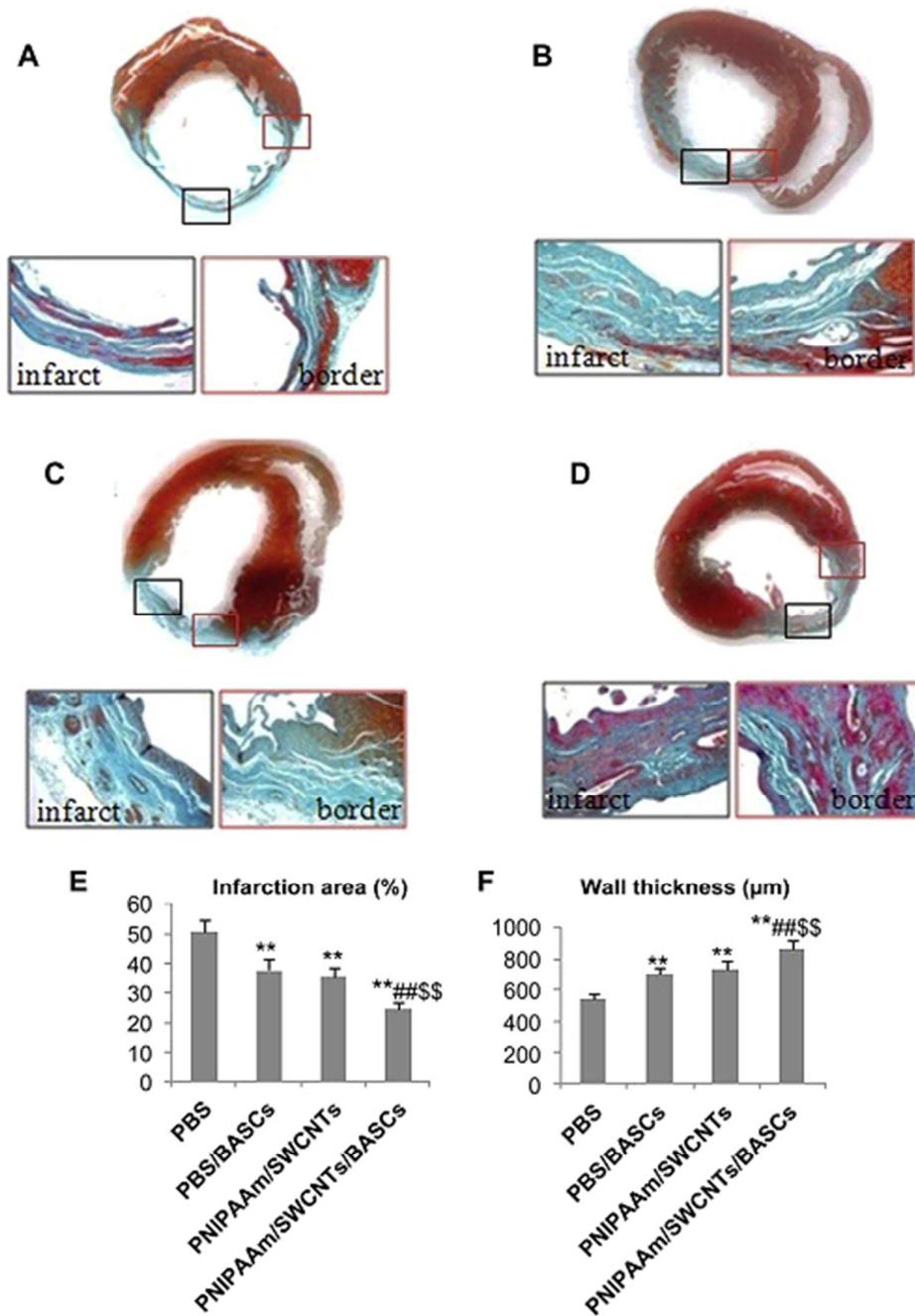


Figure 18. Infarct size and wall thickness. Cardiac structures in different groups as revealed by Masson trichrome staining 4 weeks after cell transplantation. (A) PBS group; (B) PBS/BASCs group; (C) PNIPAAm/SWCNTs group; (D) PNIPAAm/SWCNTs/BASCs group; (E) and F) Quantitative analysis of infarct size and infarct wall thickness, respectively (* $p < 0.05$

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2 compared with PBS group; **p < 0.01 versus PBS group; #p < 0.05 and ##p < 0.01
3 compared with PBS/BASCs group; \$ p < 0.05 and \$\$ p < 0.01 versus PNIPAAm/SWCNTs
4 group). Reproduced from Li et al.⁸⁶
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12 4.2 Cartilage Tissue Engineering

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14 As a connective tissue without any neural, lymphatic or vascular supply, cartilage is
15 notoriously difficult to be regenerated or reconstructed. Similar to the highly aqueous
16 environment of a cartilage tissue, hydrogels are considered suitable for use in cartilage
17 regeneration. Since the ECM of cartilage is mainly composed of proteoglycans and collagen,
18 natural polymer based hydrogels have become the first choice in cartilage tissue engineering.
19 Chitosan is a biocompatible polysaccharide with both hydroxyl and amino groups, which can
20 be chemically modified easily. Thermo-sensitive chitosan-PNIPAAm copolymers were
21 prepared by graft polymerization of NIPAAm into chitosan using ceric ammonium nitrate.
22 The copolymer showed similar sol–gel transition properties as PNIPAAm⁸⁸. After the
23 injection of the thermo-sensitive chitosan-PNIPAAm gel with MSCs into rabbit bladder wall,
24 chondrogenic differentiation of MSCs and cartilage formation were detected after 14 weeks
25 of implantation. In another study, a thermo-sensitive chitosan-Pluronic hydrogel was
26 synthesized by grafting Pluronic onto chitosan using EDC/NHS chemistry⁸⁹. This thermogel
27 has a gelling temperature of 25 °C, and a storage modulus of 10⁴ Pa, which is similar to the
28 stiffness of cartilage tissue. Cell culture studies indicated that the hydrogel could promote the
29 proliferation of bovine chondrocytes and enhance the amount of synthesized
30 glycosaminoglycan. RGD (Arg-Gly-Asp) was also conjugated onto the chitosan-Pluronic
31 copolymers by coupling the carboxyl group in the peptide with the residual amine group in
32 the copolymers⁹⁰. Conjugating RGD to chitosan-Pluronic hydrogels improved the viability
33 and proliferation of bovine chondrocytes as well as ECM expression. Ding's group
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3 synthesized an amphiphilic block copolymer, poly(ϵ -caprolactone-*co*-lactide)-PEG-poly(ϵ -
4 caprolactone-*co*-lactide), and subsequently immobilized RGD into either hydrophobic poly(ϵ -
5 caprolactone-*co*-lactide) (PCLA) blocks or hydrophilic PEG blocks⁹¹. They found that the
6 block copolymer would form a sol-gel system and the transition temperature could be tuned
7 between 26 to 40 °C. *In vitro* study showed that rat chondrocytes prefer to grow on the
8 thermogel of RGD in hydrophilic blocks rather than those in hydrophobic blocks,
9 highlighting that the influence of the immobilizing sites of RGD peptides in amphiphilic
10 polymers on the eventual cell-binding efficacy. Park et al. encapsulated Tonsil-derived MSCs
11 into a thermogelling system of PEG- poly(L-alanine-*co*-L-phenyl alanine) copolymers for 3D
12 culture⁹². To induce cell differentiation, the 3D culture system was provided with media
13 containing adipogenic, osteogenic, or chondrogenic factors. Interestingly, results showed that
14 the stem cells preferentially underwent chondrogenesis with high expressions of type II
15 collagen and sulfated glycosaminoglycan. The animal study of implantation of the hydrogel
16 into the subcutaneous layer of mice also confirmed the chondrogenesis of the cells. It was
17 suggested that the stiffness of the thermogels can provide biomechanical cues to guide the
18 differentiation of the stem cells.
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41 Mirahmadi et al. fabricated and incorporated silk fibers into thermo-sensitive
42 chitosan/glycerophosphate hydrogels to reinforce the mechanical properties of the
43 hydrogels⁹³. The silk fiber reinforced chitosan hydrogels not only showed enhanced stiffness,
44 but also supported the expression of chondrogenic phenotype for chondrocytes. Wan et al.
45 introduced amino-diethoxypropane into alginate and the modified alginate was able to form
46 hydrogel with chitosan⁹⁴. This new injectable hydrogel was investigated for cartilage
47 reconstruction by loading bone marrow mesenchymal stromal cells (BMSCs). After
48 implanting BMSCs-laden hydrogel to a rabbit knee cartilage defect model for 12 weeks,
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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 HA⁹⁵. 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 gelatine/Pluronic thermo-sensitive polymer for cartilage regeneration⁹⁶. The polymer solution showed reversible sol-gel transition behaviour at around 37 °C. Higher viability and proliferation of chondrocytes were observed in the gelatine/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 chondrocyte⁹⁷. 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 chondrocytes⁹⁸. 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 regeneration⁹⁹. 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 19).

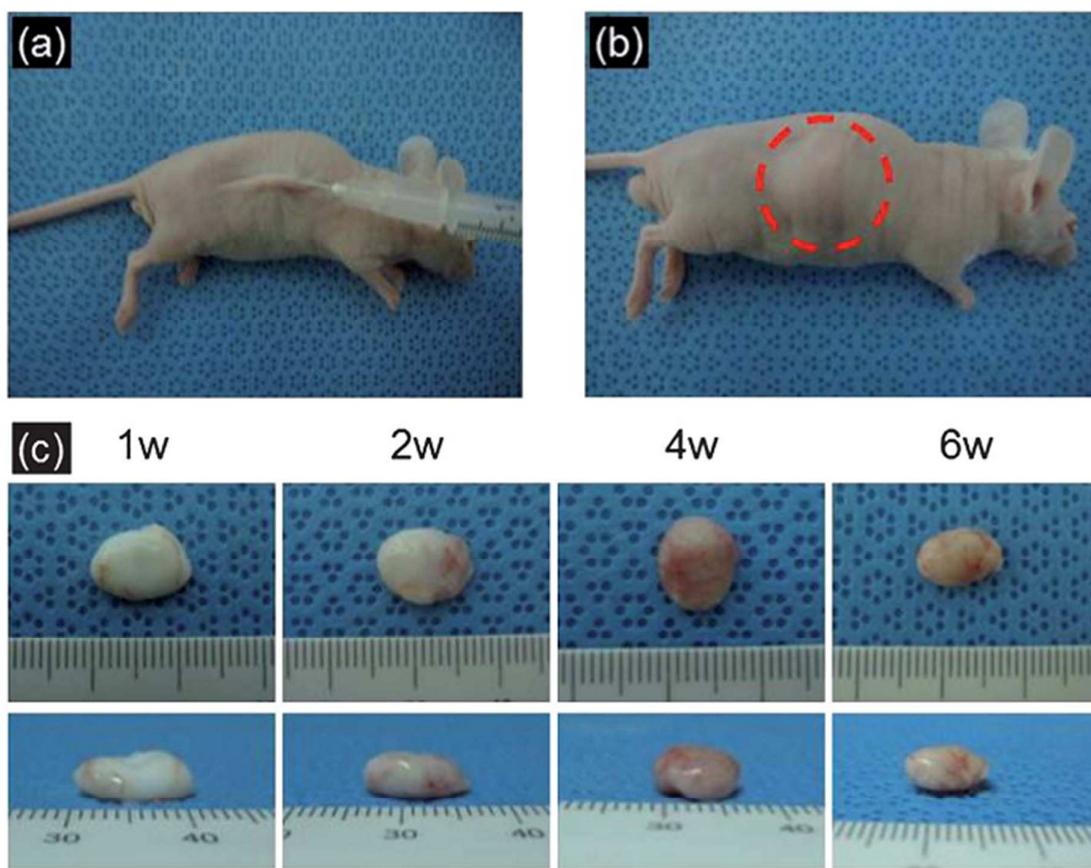


Figure 19. (a) Subcutaneous injection of chondrocyte-loaded methoxy PEG-PCL solution, (b) the formed methoxy PEG-PCL hydrogel, and (c) the hydrogels removed after 1, 2, 4 and 6 weeks. Reproduced from Kwon et al.⁹⁹

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 challenge¹⁰⁰. 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 thermo-sensitive methylcellulose as a scaffolding material for the treatment of brain defects¹⁰¹. 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 one 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 thermo-reversible hydrogels for nerve regeneration¹⁰². 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 rats¹⁰³. 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 crosslinked by telechelic difunctional PEG¹⁰⁴. 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), while the inclusion of neurosphere-like progenitors into the hydrogel resulted in a remarkable healing effect (~81 % recovery). In another study, neuronal growth factor (and

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3 brain derived neurotropic growth factor) loaded microspheres were incorporated into a PEG-
4 poly(L-alanine) thermogelling system for 3D cell culture¹⁰⁵. Tonsil-derived MSCs were
5 seeded in the hybrid system for neuronal differentiation. After 2 weeks of culture, the stem
6 cells underwent multipolar elongation initially, followed by upgraded expressions of the
7 neuronal biomarkers such as nuclear receptor related protein (Nurr-1), neuron specific
8 enolase, microtubule associated protein-2, neurofilament-M, and glial fibrillary acidic protein
9 in both mRNA level and protein level. The promoted neuronal differentiation of tonsil-
10 derived MSCs was attributed to the suitable modulus of the thermogel (~800 Pa similar to the
11 stiffness of brain tissue) and sustainable stimulation of growth factors released from the
12 microspheres.
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15 Collagen constitutes more than 50 % of nerve ECM, and thus collagen and its derivatives
16 have been widely evaluated as biomaterials for nerve guide applications. A nerve tissue
17 engineering scaffold consisting of PLLA fibers and thermo-sensitive collagen hydrogel was
18 designed to improve the construction of peripheral nerve¹⁰⁶. Instead of using a static culture,
19 dynamic culture was performed for bone marrow MSCs at an oscillating frequency of 0.5 Hz
20 and 35° swing angle above and below the horizontal plane. The thermo-sensitive collagen
21 hydrogel under dynamic culture enhanced the viability of the grafted cells and minimized cell
22 loss during the initial implantation stage. Cheng et al. developed a thermo-sensitive
23 gelatin/chitosan/glycerol phosphate hydrogel as a cell carrier for nucleus pulposus (NP)
24 regeneration¹⁰⁷. To overcome cell death caused by oxidative stress, ferulic acid (a Chinese
25 herb medicine) was added into the hydrogel system for antioxidant and anti-inflammatory
26 properties. Nucleus pulposus cells were submitted to oxidative stress caused by H₂O₂
27 treatment. Cells cultured on the FA-containing hydrogel exhibited down regulation of MMP-
28 3 and hence apoptosis inhibition. Moreover, the thermo-sensitive hydrogel promoted NP
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2 regeneration by up-regulating mRNA levels of aggrecan and type II collagen, as well as
3 increasing the production of sulfated-glycosaminoglycan.
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10 **4.4 Thermogels for Other Bio-applications**

11 Post-operative intestinal adhesion is a common complication in surgery, and thermoreversible
12 hydrogels, with biodegradable properties and unique sol-gel transition capability, could serve
13 as a promising anti-adhesion material. A thermogel system of PCLA-PEG-PCLA block
14 copolymers was employed as a barrier material for prevention of post-operative intestinal
15 adhesion in a rabbit model¹⁰⁸. The hydrogel showed good biodegradability *in vivo* and its
16 integrity could be retained for as long as several weeks. The *in vivo* study also proved that the
17 thermogel system effectively reduced the formation of intraperitoneal post-operative
18 intestinal adhesion even after 30 days. It was also reported that loading RGD molecules into
19 PCLA-PEG-PCLA thermogel could result in a better performance in anti-adhesion
20 properties¹⁰⁹. In the system, the hydrogel afforded a physical barrier and the encapsulated
21 RGD acted as an integrin blocker to enhance the anti-adhesion. Similarly, a PLGA-PEG-
22 PLGA thermogel was synthesized as a barrier to prevent spinal epidural fibrosis in a
23 postlaminectomy rat model¹¹⁰. Results showed that the thermogel effectively reduced
24 epidural scarring, and prevented the subsequent adhesion to the dura mater with improved
25 performance as compared to the positive control, chitosan gel. Yu et al. investigated the
26 efficacy of three different PEG/polyester thermogels [PLGA-PEG-PLGA, PCL-PEG-PCL
27 and poly(ϵ -caprolactone-*co*-D,L-lactic acid)-PEG-poly(ϵ -caprolactone-*co*-D,L-lactic acid)
28 (PCGA-PEG-PCGA)] for preventing post-operative abdominal adhesion in a rabbit model of
29 sidewall defect-bowel abrasion¹¹¹. They found that PLGA-PEG-PLGA showed the best
30 prevention of abdominal adhesions, probably due to their suitable viscoelastic properties
31 (phase angle = ~45°) and excellent biodegradable rate.
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2 Jeong's group investigated the hepatogenic differentiation of tonsil-derived MSCs by using
3 the 3D thermogelling matrixes of PEG-poly(L-alanine) diblock polymer¹¹². The 6 wt%
4 thermogel showed the modulus of ~1000 Pa at body temperature, which is similar to the
5 stiffness of the decellularized liver tissue. Co-cultured with hepatogenic growth factors for 4
6 weeks, the stem cells expressed hepatogenic genes (such as albumin, cytokeratin 18 and
7 hepatocyte nuclear factor 4α) and exhibited typical metabolic behaviour of hepatocytes (such
8 as uptake of cardiogreen and low-density lipoprotein). An interesting work involving the use
9 of an injectable PLGA-PEG-PLGA triblock copolymer thermogel system for sustained
10 intravitreal delivery of dexamethasone was conducted¹¹³. Compared to a dexamethasone
11 suspension, the thermogelling systems showed increased intravitreal retention time from
12 hours to over 1 week. Moreover, the implantation of the thermogels did not impair the
13 morphology of retina and cornea.
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32 **5. Lessons from the development of Oncogel: From initial concept to clinical trials**
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34 Limitations associated with systemic administration spurred the development of
35 localised/targeted delivery vehicle. One such example is OncoGel, an experimental localised
36 drug delivery system that allows the controlled release of paclitaxel (an approved intravenous
37 anticancer drug) from thermo-sensitive ReGel polymer. The development of Oncogel
38 evolved from the conception of reverse thermal gelation of biodegradable polymers
39 consisting of A and B blocks arranged as BAB or ABA. Block A is poly(lactide-co-
40 glycolide) (PLGA) and block B is poly(ethylene glycol) (PEG)¹¹⁴. In 2001, MacroMed Inc.
41 (Salt Lake City, Utah, USA) developed a thermal gel depot-based delivery system –
42 ReGel¹¹⁵. This ABA-type biodegradable thermogel demonstrates reverse thermal gelation
43 property where a water-soluble aqueous solution of the polymer spontaneously transforms to
44 a water-insoluble gel at body temperature. This brought about the development of OncoGel
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(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 longer¹¹⁶. Both *in vivo* and *in vitro* release studies demonstrated the biocompatibility of OncoGel and the continuous release of paclitaxel concurrent with degradation over 4 to 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 therapies¹¹⁸. 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 thermo-responsive 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 month¹¹⁵. 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 four 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 tumors¹²⁰. Paclitaxel exhibits anti-cancer activity through a number of mechanisms: mitotic inhibitor (microtubule stabilization), anti-angiogenic agent and radiation sensitizer¹²¹. Prasad et al. has summarized the development of OncoGel in a flowchart (Figure 20)¹²².

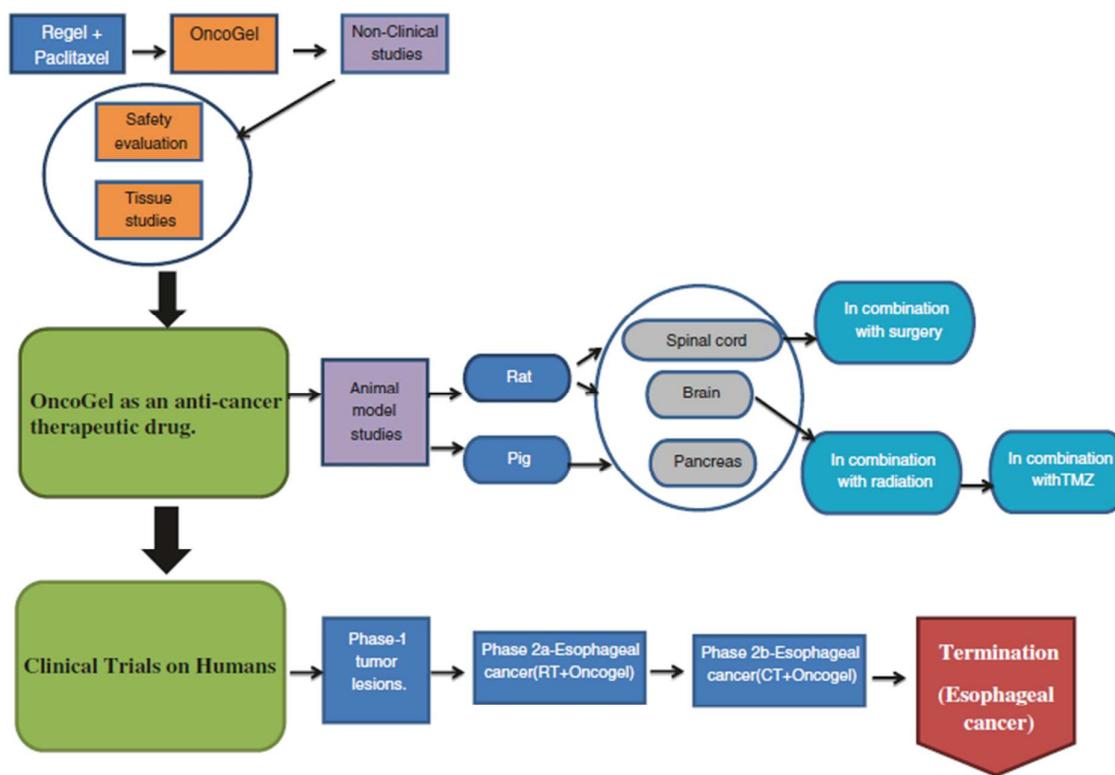


Figure 20. Flowchart of the development of Oncogel. Reproduced from Prasad et al.¹²²

5.2 Mechanistic principles on non-clinical 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 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 day¹¹⁵. 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, 117b, 123}. An ADME study

(absorption, distribution, metabolism and excretion) of ^{14}C paclitaxel on breast tumor xenograft in mice over 42 days following OncoGel administration intralesionally showed ^{14}C paclitaxel localized within the tumor, with minimal levels (< 0.2%) detected in blood, tissues or urine¹¹⁵. Efficacy of OncoGel (6 mg/ml paclitaxel) against human tumor xenograft (MDA231) is comparable with the maximum tolerated systemic dose (ten-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 two days of dosing¹¹⁵.

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 fourteen 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

glioblastoma¹²⁵. Efficacy of Oncogel plus TMZ plus RT resulted in 100% long term survival, indicating strong therapeutic effect (Figure 21)¹²⁶.

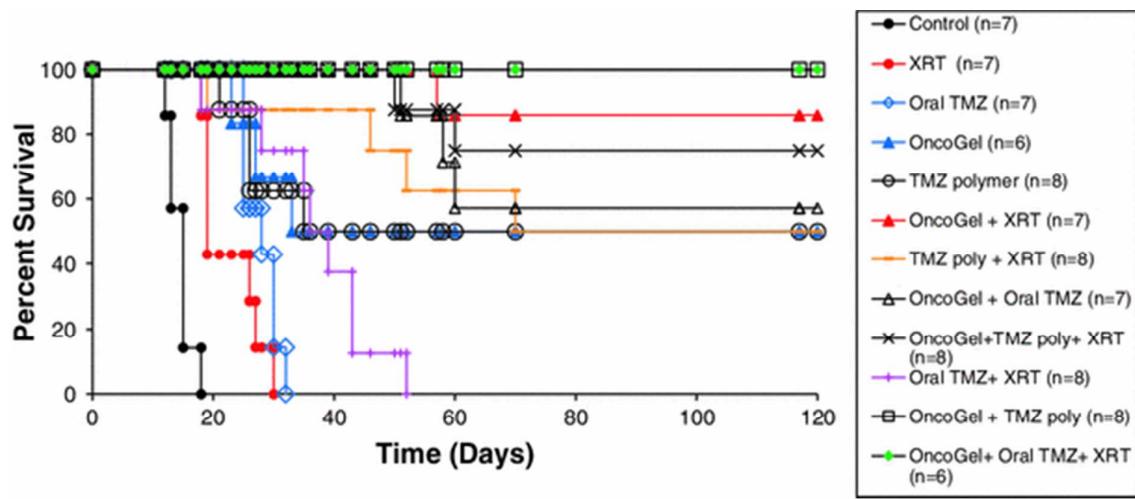


Figure 21. Intracranial efficacy of TMZ given IC or PO in combination with OncoGel 6.3 mg/ml with and without XRT for the treatment of experimental malignant gliosarcoma. F344 rats were intracranially implanted with 9L tumor. Controls ($n = 7$) received no further treatment (*filled circle*) and had a MS of 15 days. Animals receiving XRT (20 Gy) on Day 5 ($n = 7$) (*filled circle*) and animals that received oral TMZ on Days 5–9 ($n = 7$) (*open diamond*) had MS of 19 and 28 days, respectively. Animals receiving OncoGel 6.3 on Day 0 ($n = 6$) (*filled triangle*) had a MS of 33 days. Animals receiving a TMZ polymer on Day 5 ($n = 8$) (*open circle*) had a MS of 35 days. Animals that received OncoGel 6.3 and XRT ($n = 7$) (*filled triangle*) did not reach MS with 85 % long term survivors (LTS). Animals that received a TMZ polymer and XRT ($n = 8$) (*emdash line*) reached MS on Day 70 with 50 % LTS. Animals that received OncoGel6.3 and Oral TMZ ($n = 7$) (*open triangle*) did not reach MS with 57 % LTS. Animals receiving OncoGel 6.3, TMZ polymer and XRT ($n = 8$) (*cross symbol*) did not reach MS with 75 % LTS. Animals that received Oral TMZ and XRT ($n = 8$) (*vertical line*) had a MS of 35 days. Animals that received either OncoGel 6.3 and TMZ

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2 polymer ($n = 8$) (*open square*) or the triple combination of OncoGel 6.3, oral TMZ and XRT
3 (n = 6) (*filled circle*) had no deaths with both groups having 100 % LTS. Reproduced from
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5 Vellimana et al.¹²⁶
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10 These studies revealed that OncoGel used as adjuvant treatment prior to surgery may provide
11 tumor shrinkage, or if used after surgery may slow or prevent tumor regrowth. This gave the
12 impetus for evaluating OncoGel in clinical trials. Phase 1 study characterized the toxicity,
13 pharmacokinetics and preliminary antitumor activity associated with OncoGel administered
14 directly into solid tumors^{118b}. OncoGel delivered intralesionally at doses up to 2.0 mg
15 paclitaxel/cm³ tumor volume was well tolerated and the paclitaxel remained localized at the
16 injection site. Systemic exposure of the drug was minimized. Phase 2a was a dose-escalation
17 study evaluating the toxicity, pharmacokinetics and preliminary antitumor activity of
18 OncoGel plus RT therapy in patients with inoperable esophageal cancer^{118a}. OncoGel given
19 as an adjunct to RT was well tolerated in patients with inoperable esophageal cancer and
20 provided prolonged paclitaxel release with minimal systemic exposure^{118a}. OncoGel plus RT
21 seemed to reduce tumor burden as evidenced by dysphagia improvement, tumor size
22 reduction, and negative esophageal biopsies. These promising data spurred clinical
23 development. Phase 2b clinical trial was conducted by combining OncoGel and
24 chemoradiotherapy for 154 randomized esophageal cancer patients subsequently undergoing
25 surgery, OncoGel/Chemoradiotherapy (CRT) (n=78) or CRT alone (n=76)¹²⁷. Combination
26 of intratumoral OncoGel injection and CRT was well tolerated, without a notable increase in
27 systemic side effects. However, OncoGel failed to demonstrate enhancement in efficacy of
28 chemoradiotherapy in localized delivery of paclitaxel compared with systemic administration.
29 Oncogel was then terminated as a potential therapy for esophageal cancer in 2010¹²⁸.
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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 2¹²⁹ include hormone therapies, signal transduction inhibitors, gene expression modulator, apoptosis inducer, angiogenesis inhibitor, immune modulator, and toxin delivery molecules.

Table 2. Summary of FDA approved targeted cancer therapies¹²⁹

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-trastuzumab emtansine (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™)

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 multi-pronged 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 non-clinical 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 trials¹³⁰. Clearer conclusions drawn for paclitaxel transport mechanism in brain tissue could be applied for other tissue modelling. 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 perivascular delivery of dipyridamole¹³¹. 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 release⁷³. The GLP-1 released from ReGel formulation *in vitro* and *in vivo* showed constant release for two weeks with no initial burst release. Animal study demonstrated that the plasma insulin level was increased, and the blood glucose level was controlled for two 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

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2 application¹³². More studies can be done to include injection of human-derived tumor cells
3 into immune-compromised animal hosts to overcome limitations of using non-human cell
4 lines. The goal is to attain paclitaxel responses which will be more relevant to the clinical
5 setting. A more accurate re-creation of the tumor microenvironment allows a more accurate
6 understanding of drug delivery mechanism before clinical studies¹²⁴.
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15 The primary application for Oncogel would be for inoperable tumors. This minimally
16 invasive Oncogel treatment is a less preferred approach than open surgery because patients
17 do not wish to wait for gradual tumor regression over time¹³³. Hence, apart from performance
18 aspects of drug delivery systems, patients' preferences and perspectives should be noted
19 during clinical trials.
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30 **6. Outlook and Perspectives**

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32 The numerous innovative research works cited here show a spectacular evolution of
33 thermogel technology in recent decades. Thermogels can be effectively used for delivery and
34 encapsulation of active ingredients such as bioactive drugs, genetic material, cells and
35 proteins. This administration method is very convenient and non-invasive because while the
36 gel is fluid during mixing and injection, it undergoes sol-gel transition at the target site under
37 physiological conditions. Copolymerization with labile groups and end-group
38 functionalization can provide biodegradability after the goal of an implant is accomplished.
39 The lack of popularity of Oncogel has spurred an evaluation of therapeutic methods using
40 patients' perspectives. The challenge with Oncogel was that most patients would rather have
41 an open surgery than to wait for tumor size reduction via a gel release system¹³³. Hence the
42 potential application of Oncogel is changed to inoperable tumors. Identifying the right
43 application is thus essential to the success of thermogels.
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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 non-toxic 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 anti-oxidant moieties such as vitamin E, ferulic acid and ascorbic acid may help to reduce oxidative stress, while 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 by-products should be considered during the material-design stage. With the thermo-sensitive 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 thermo-sensitive materials remains unexplored (Table 1).

Thermo-responsive 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.

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