Biodegradable Thermogelling Polymers: Working Towards Clinical Applications

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Biodegradable Thermogelling Polymers: Working Towards Clinical Applications

*Qing Qing Dou, Sing Shy Liow, Enyi Ye, Rajamani Lakshminarayanan, and Xian Jun Loh**

As society ages, aging medical problems such as organ damage or failure among senior citizens increases, raising the demand for organ repair technologies. Synthetic materials have been developed and applied in various parts of human body to meet the biomedical needs. Hydrogels, in particular, have found extensive applications as wound healing, drug delivery and controlled release, and scaffold materials in the human body. The development of the next generation of soft hydrogel biomaterials focuses on facile synthetic methods, efficacy of treatment, and tunable multi-functionalities for applications. Supramolecular 3D entities are highly attractive materials for biomedical application. They are assembled by modules via various non-covalent bonds (hydrogen bonds, p–p stacking and/or van der Waals interactions). Biodegradable thermogels are a class of such supramolecular assembled materials. Their use as soft biomaterials and their related applications are described in this Review.

1. Introduction

Biodegradable materials have found extensive applications in the medical field. These materials are applied in various applications ranging from those requiring high strength and modulus such as orthopedic applications, to softer and delicate applications such as ophthalmological applications. The principle of using biodegradable materials is that the degradation debris of these materials can be automatically cleared off the body after their period of use. The small pieces of degradation debris are subsequently excreted out of the body, removing these biodegradable polymers seamlessly. Supramolecular chemistry can be exploited to enhance material properties such as the modulus of the hydrogel or to fine tune the release profile of bioactive components in the gel matrix. This can be achieved by assembling molecular components with specific structures and/or functionalities via non-covalent chemical interactions.[1,2] The use of weaker and dynamic non-covalent interactions between molecules leads to the development of reversible and functional materials, which can respond to a variety of stimuli such as light, heat, enzymes, and pressure.[3–7] Over the last decade, several types of molecular assemblies have been used to develop stimuli-responsive hydrogels.[3,8–10] These include host–guest assembled polymers, metallo-crosslinked gels, and thermogelling polymers. Thermogelling copolymer systems are a class of soft materials, which assemble via supramolecular interactions of the polymeric blocks. The aqueous polymeric solution of this material exists as low viscosity fluids at low temperatures and solidifies into a hydrogel at elevated temperatures, typically body temperature. While this change seems to be contrary to the conversion of water to ice, the mechanism is totally different from the phase change in water. The reversible conversion between the sol and gel states can be understood as the hydration and dehydration of polymer chains. A thermogelling copolymer is an amphiphilic macromolecule with finely tuned balance of hydrophobicity and hydrophilicity. At lower temperature, hydrogen bonding between water molecules and polymers keeps the polymer solvated in aqueous solution. As the temperature is raised, the hydrogen bonds are weakened by random thermal motion of the polymer molecules and the water–polymer interactions are weakened. The association of the hydrophobic components leads to the formation of nano-domains, which serve as crosslink points and induces a sol-to-gel change. The gelation of the polymer solution is concentration dependent. When the concentration of the polymer is low, the copolymer chains self-assemble into aggregated micelles when the temperature is raised. These micelles have hydrophilic segments presented in the corona and hydrophobic segments in the core. As the concentration of the polymer (and consequently micelles) increases, the micelles are packed closer together. The critical concentration at which the micelles pack close enough together to induce a gel state is known as the critical gelation concentration (CGC).

This unique property of thermally induced gelation of polymer solution is especially useful for drug delivery and other similar clinical applications. In a typical application of this system in the biomedical context, the molecules for treatment such as peptides or drugs can be mixed with the

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polymer solution in a sol state followed by injection to the targeted area. Upon exposure to body temperature, the gelation of polymer starts due to temperature increment and the in situ-molded flexible hydrogel acts as a sustained drug delivery depot. Besides small molecules, this system also suited for the delivery of cells. For the advanced delivery of cells to the body, the polymer can be molded into the appropriate shape in the application site and can act as a 3D tissue regeneration scaffold for cell proliferation. This system presents obvious advantages. First, peptide formulations can be prepared in aqueous solutions at low temperature, avoiding peptide denaturation due to either the interactions of organic solvents or high temperatures. Second, the thermogelling polymers can be easily administrated by injection, and the polymer solution can be pre-sterilized by syringe filtration. Third, the high water content of the gel matrix results in improved compatibility with injection site. Last, when incorporated with biodegradable segments, the thermogelling polymers can be removed from the body via excretion after its intended purpose is achieved. The ease of usage as well as the key advantages of this material makes it a very exciting material for biomaterial applications. There are many significant reviews in literature that examines the different aspects of thermogelling polymers. Lee et al. discussed the material characteristics of thermogels, particularly on pH-/temperature-sensitive sol–gel transition and polyesters-based thermogels.[11] Ding et al. provided a comprehensive summary of the different types of thermogelling polymers and focused on polyester thermogels.[12] Finally, a recent review by Jeong et al. summarizes the different types of thermogelling materials focusing on material characteristics and mechanism of sol–gel transition.[13] The literature reviewed here is not meant to be exhaustive but rather meant to provide the reader with a general guide of this research area, discussing the methods of preparation of the thermogels with an eye towards applications in the biomedical field.

2. Preparation of Thermogelling Polymers

2.1. Ring-Opening Polymerization

Biodegradable polymers such as poly(ε-caprolactone) (PCL) and poly(lactic acid) (PLA) are typically synthetically prepared by ring-opening polymerization of lactones. The basic monomeric unit of PLA is lactic acid. In theory, the esterification of lactic acid forms PLA and the reaction can be expected to be straightforward. However, this is an equilibrium reaction and it is difficult to remove the water produced by the esterification process completely. The bonds formed are easily hydrolyzed by the water molecules and the maximum attainable molecular weight is thus limited. This problem is solved by using a cyclic lactide. This lactide undergoes a ring-opening polymerization to give PLA. As there is no water molecules produced in the course of the reaction, the molecular weights that can be attained are much higher than typical polycondensation reactions. Ring opening polymerization has been used by Jeong et al. to prepare thermogelling polymers. Using a telechelic dihydroxyl compound as the initiator, either a small molecule glycol or poly(ethylene glycol), many polyesters can be synthesized in this manner. In particular, polymers such as PCL,[14–17] PLGA,[18–26] and PLA[27,28] have been reported. Poly(amino acids) have also been polymerized by ring-opening polymerization of *N*-carboxyanhydrides (NCA). To date, poly (L-Ala-*co*-L-Phe)-poly(propylene glycol)-poly (ethylene glycol)-poly(propylene glycol)-poly(L-Ala-co-L-Phe), poly(ethylene glycol)-poly(L-alanine-co-L-phenyl alanine), poly-(ethylene glycol)-poly(D-alanine-co-D-phenyl alanine), poly(ethylene glycol)-poly(L-alanine-co-L-phenyl alanine) grafted chitosan have been demonstrated to have a thermogelation effect.[29–33]

2.2. Radical Polymerization

Radical polymerization is the workhorse of many polymer chemists and has been used extensively to fabricate materials, which are easily scalable and also having multiple characteristics due to the different monomers in the polymer backbone. Typical monomers polymerized include *N*-isopropylacrylamide (NIPAAm) and poly(acrylic acid) (PAA).[34,35] Liu et al. reported the free radical polymerization of PNIPAAm grafted to methylcellulose (MC) using ammonium persulfate and *N,N,N′,N′*-tetramethyl ethylene diamine as an initiator.[36] The sol–gel transition of the thermogels occurs within a minute with tunable transition temperature based on the composition of the copolymer. Recently, controlled radical polymerization techniques such as atom transfer radical polymerization (ATRP) have allowed for new block architectures as well as modulation of the thermogel behavior. Recently, Kitazawa et al. reported the synthesis of a thermosensitive triblock copolymer, consisting of poly(benzyl methacrylate) as the terminal blocks and poly(methyl methacrylate) as the middle block, by ATRP.[37] This polymer exhibits a thermogelling effect in an ionic liquid and shows an extremely high gelation temperature of above 100 °C. Abandansari et al. reports the synthesis of a pentablock polymer of PNIPAAm-PCL-PEG-PCL-PNIPAAm, which was synthesized by combining ring-opening polymerization and ATRP.[38] Li et al. synthesized a class of thermogels for the delivery of cardiosphere-derived cells (CDCs).[39] The hydrogels were based on a central polycaprolactone unit with flanking PNIPAAm, poly(2-hydroxyethyl methacrylate) and poly(dimethyl-γ-butyrolactone acrylate) (**Figure 1**). Atom transfer radical polymerization was utilized for the fabrication of well-defined thermogelling polymers. These polymer solutions formed semi-solid gels within 5 s.

2.3. Formation of Poly(urethane)s

The formation of multiblock copolymers with high molecular weights is the basis for the preparation of thermogelling copolymers with extremely low gelation concentrations. Typically, this is prepared by a one-pot reaction of poly(ethylene glycol) as the first block, poly(propylene glycol) as the second block, and a third block polymer of choice. Without the third block, the thermogelling effect is also observed.[40] However, based on the different requirements such as degradation stability, hydrophobicity of the block copolymer, and drug release profile of the thermogel, the third block can be used to

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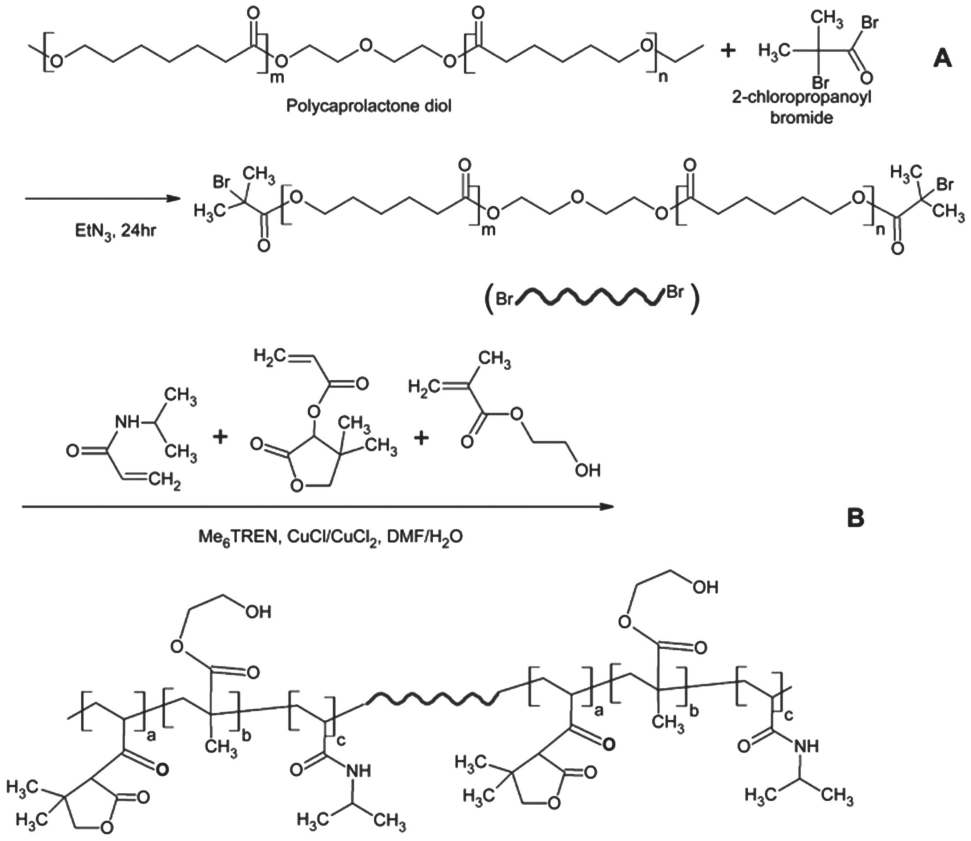

Figure 1. Synthesis of A) difunctional polycaprolactone initiator and B) hydrogel polymers by ATRP. Reproduced with permission.[1] Copyright 2011 Elsevier.

tune the thermogel properties. Over the past 7 years, several blocks have been utilized including poly[(R)-3-hydroxybutyrate] (PHB),[41–44] PLA,[45] PCL,[46,47] poly(ethylene butylene),[48,49] and poly(trimethylene carbonate).[50] Recently, Park et al. reported the synthesis of thermogelling polymers with functional groups in the backbone to allow for bio-functionalization to enhance cell–materials interactions.[51] Here, an amine-functionalized ABA block copolymer, poly(ethylene glycol)-poly(serinol hexamethylene urethane) (ESHU) with one free primary amine group on every repeating unit was synthesized. The polyurethane unit functions as the hydrophobic block and PEG acts as the hydrophilic block. Serinol is a serine derivative with two hydroxyl groups and one amine group; the hydroxyl groups allow for conjugation with the isocyanate groups to form the urethane linkages and the amino group can be used for further functionalization. The amino group was first protected during the urethane formation. This protected amino group was reacted to form a bio-functionalized ESHU with IKVAVS peptide which shows rapid thermogelling property.

2.4. Chemical and Physical Modifications of Natural Polymers

Many chitosan-based thermogelling systems have been previously reported. These include combinations of chitosan and glycerol-phosphate, poly(ethylene glycol)-grafted chitosan, poly(vinyl alcohol)/chitosan blended hydrogels.[52–55] Many different varieties of thermogels can be prepared, however, complicated multi-step reactions using organic solvents are not preferred as these materials may not be very compatible for biomedical applications. Chitosan itself is also poorly water soluble under physiological conditions making it difficult to formulate for drug delivery applications. Simple mixing reactions or one-step chemical modification methods are ideal for clinical applications. For example, a chitosan-ammonium hydrogen phosphate (chitosan-AHP) blend was found to have thermogelling properties (**Figure 2**).[56] The preparation was simple as shown in the following. Chitosan was first dissolved in mild acetic acid solution. Following that, the chitosan solution was mixed with ammonium hydrogen phosphate solution to yield the thermogel.

Another simple formulation method relies on the modification of glycol chitosan. Glycol chitosan is water soluble under physiological conditions due to glycol residues. Its chitosan backbone is the reason behind the non-cytotoxic and biodegradable nature of the material. A glycol chitosan-based thermogelling polymer was recently prepared as a thermogelling polymer (**Figure 3**).[57] Here, selective *N*-acetylation of glycol chitosan was carried out in a mixed solvent of water and methanol. Acetic anhydride was added into the glycol chitosan solution for the acetylation of glycol chitosan. The polymer was treated with sodium hydroxide solution for the removal of the O-acetylated moieties and purified by dialysis.

Besides chitosan, naturally derived polymers such as hyaluronic acid can be chemically modified to form thermogels. For

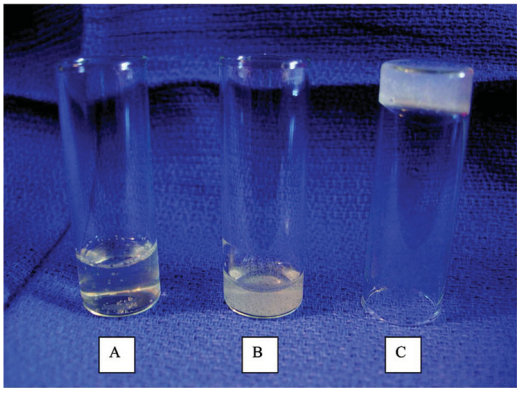

Figure 2. Photograph showing the thermogelation of chitosan–AHP solution. A) Chitosan solution in 0.5% acetic acid. B) Chitosan–AHP solution (0.075 g of AHP added to 5 mL of chitosan solution in an ice bath and stirred for 2 min followed by incubating at 37 °C for 4 min. C) Thermogelled chitosan–AHP solution after incubating the chitosan–AHP solution at 37 °C for 11 min. Reproduced with permission.[56] Copyright 2007, American Chemical Society.

example, copolymers of NIPAAm and acrylic acid *N*-hydroxysuccinimide were first synthesized via free radical polymerization and then bonded to amine-functionalized hyaluronic acid (HA) to form a thermogelling polymer.[58]Other types of naturally derived polymers such as methyl cellulose have well-documented thermogelling effect as well.[59]

3. Applications of Thermogelling Polymers

Thermogelling polymers can be formulated with various biomolecules in solution and encapsulated in the gel matrix upon temperature-induced physical gelation. This unique property renders them suitable for numerous biomedical implications. Applications of thermogelling polymers date back to the 1980s.[60] Since then, they have been widely applied in various biomedical applications, such as drug delivery and controlled drug release,[61–63] tissue engineering,[64–68] eye treatment, and eye care applications.[69,70]

3.1. Drug Delivery and Controlled/Sustained Drug Release

3.1.1. Cancer Treatments

Drug delivery is the most explored application for thermogelling polymers. Numerous groups have invested significant efforts to design thermogelling polymers for this purpose. The capability of delivery has been greatly improved and the period of controlled release has been extensively prolonged. Our group has developed a series of thermogelling polymers for controlled drug release.[40–42,45–50,71–73] Cancer is one of the critical ailments, which urgently require technological intervention. As a result, anticancer drugs such as doxorubicin and paclitaxel were studied as potential candidates for encapsulation. A combination therapy of local cancer radiotherapy was combined with chemotherapy using a therapeutic radioactive nuclide (Re-188-Tin colloid) and a chemotherapeutic drug (liposomal doxorubicin) encapsulated in thermogelling PCL-PEG-PCL copolymer.[74] Sustained release of the radioactive nuclide and doxorubicin from the thermogel was slow lasting for at least 10 d. Intratumoural administration of the loaded thermogel in mice with hepatocellular carcinoma (HCC) was carried out. Its retention by the tumor, spatiotemporal distribution, and therapeutic effect were evaluated. The gel depot held the radioactive nuclide inside the tumor, whereas free radioactive nuclide diffused rapidly from the tumor. The tumor growth was more profoundly inhibited by up to 80% on day 32 by treatment with the loaded thermogel. This thermogel offers the advantage of focusing radiotherapy and chemotherapy locally to maximize their effects on hepatocellular carcinoma. A paclitaxel/thermogel formulation known as OncoGel has been extensively studied for cancer therapeutics. In this case, the thermogel was a PLGA–PEG–PLGA triblock copolymer with molecular weight of about 4 kDa.[75] This copolymer was able to solubilize very hydrophobic drugs and sustained the release of paclitaxel for approximately 50 d. When OncoGel was directly injected into the tumor, slow clearance of paclitaxel from the injection site was observed, the increased efficacy of this formulation compared to pure drug was also observed. Endoscopic ultrasound-guided injection of OncoGel was carried out into the pancreas of pigs to study the application of this formulation for the minimally invasive local treatment of unresectable pancreatic cancer.[76] After injection, no side effects (fever, vomiting, or anorexia) were observed in the pigs. The gel depot was stable and remained in the tail of the pancreas, showing that this gel formulation could be used as a sustained delivery device for paclitaxel. OncoGel was also used for the study of treatment in a rat metastatic spine tumor model,[77,78] inoperable esophageal cancer,[79] for administration onto superficially accessible progressive solid cancerous lesions where no therapeutic treatment was available[80] and treatment of brain tumors.[81] Our group has published a series of multiblock poly(ether carbonate

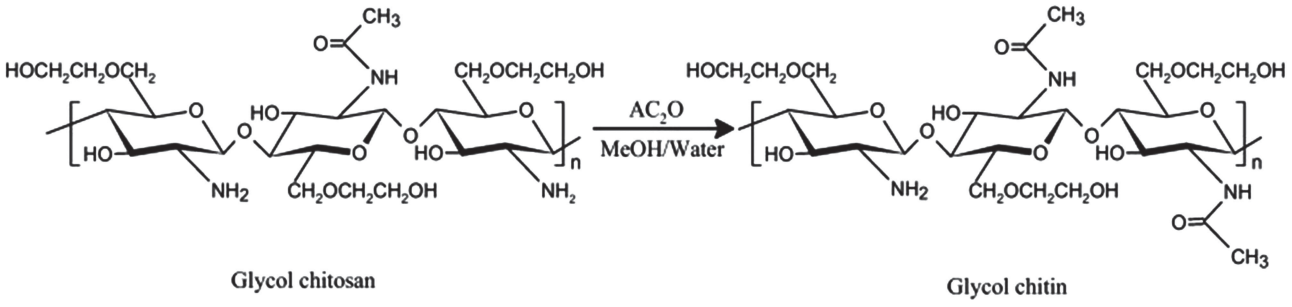

Figure 3. Synthesis scheme of glycol chitin. Reproduced with permission.[57] Copyright 2013, Elsevier.

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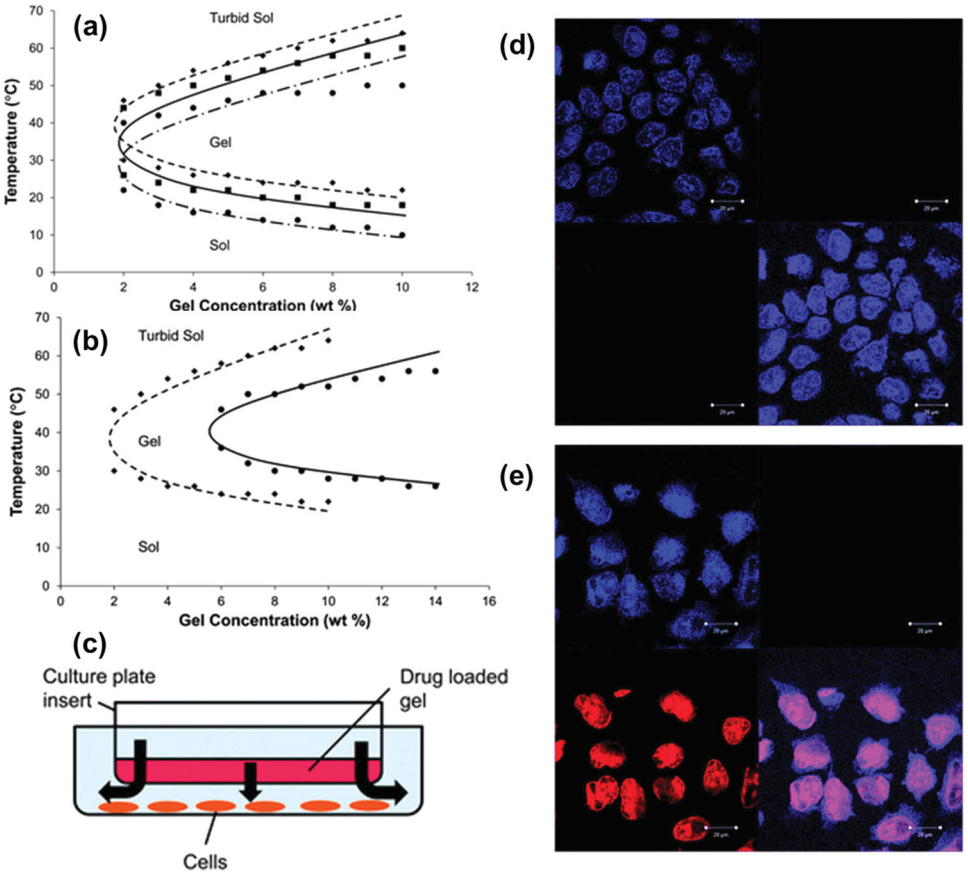

Figure 4. a) Effect of PTMC content on the sol–gel phase diagrams of poly(PEG/PPG/PTMC urethane) in aqueous solution (Dashed line: P1 (2.6 wt% PTMC), solid line: P2 (5.1 wt% PTMC), and dash-dot line: P3 (7.8 wt% PTMC); b) Effect of polymer molecular weight on the sol–gel phase diagrams of poly(PEG/PPG/PTMC urethane) in aqueous solution. Dashed line: (molecular weight = 57 900 g mol^{-1}) and solid line: (molecular weight = 26 700 g mol^{-1}); c) Experimental setup for the testing of the toxicity of the drug-loaded thermogels on HeLa cells. The black arrows indicate the free diffusion of the drug into the culture medium; d,e) CLSM images of HeLa cells incubated for 5 d under different conditions: d) control (free doxorubicin solution), and e) doxorubicin released from poly(PEG/PPG/PTMC urethane) thermogel. Reproduced with permission.[50] Copyright 2012 Royal Society of Chemistry.

urethane)s were synthesized with poly(trimethylene carbonate), poly(ethylene glycol), and poly(propylene glycol) segments (**Figure 4**). Gelation of the polymer could be realized at concentrations as low as 2 wt%, the gelation concentration and gel transition temperature can be varied by changing the composition and the molecular weight of the copolymer. A sustained release of over 50 d was achieved from the doxorubicin-loaded gels. The growth of HeLa cells was significantly controlled when cultured with doxorubicin-loaded gels compared with free doxorubicin solution.[50]

3.1.2. Protein or Drug Delivery

Not limited to therapeutic drugs, thermogelling polymers were also used for the delivery of proteins, such as lysozyme and insulin.[82] PLGA/PEG graft copolymers were studied for diabetes control by sustained insulin delivery.[83] An injection of the thermogel/insulin formulation controlled the blood glucose level in diabetic rats from 5 to 16 d. Bhattarai et al. reported an injectable chitosan-based thermogel synthesized by grafting PEG onto a chitosan backbone.[52] Release of a model protein, bovine serum albumin (BSA), was achieved over 70 h. Extended release of BSA, of up to 40 d was achieved by crosslinking the thermogel with genipin. Lee et al. presented a pH- and temperature-responsive albumin-linked poly(amino urethane) multiblock copolymers were developed for long-term protein delivery.[62] The gel window was finely tuned to match the physiological condition, which allowed the smooth flow of polymer solution to the back of the Sprague–Dawley rats (**Figure 5**). After it formed the solid gel at body temperature, the sustained release of lysozyme was observed over 4 weeks in the in vitro condition and over 2 weeks in the in vivo conditions, respectively. Ding et al. reported the use of a thermogel for the delivery of exenatide for the treatment of type II diabetes.[84] The PLGA–PEG–PLGA triblock copolymer thermogel was found to reduce the rate of degradation of the polypeptide significantly. The initial burst release was circumvented by the addition of zinc acetate to slow down the exenatide release by formation of insoluble Zn-exenatide complexes in the gel matrix. In vivo experiments established an improved glucose tolerance in mice for a week after a single subcutaneous injection of the optimal exenatide formulation.

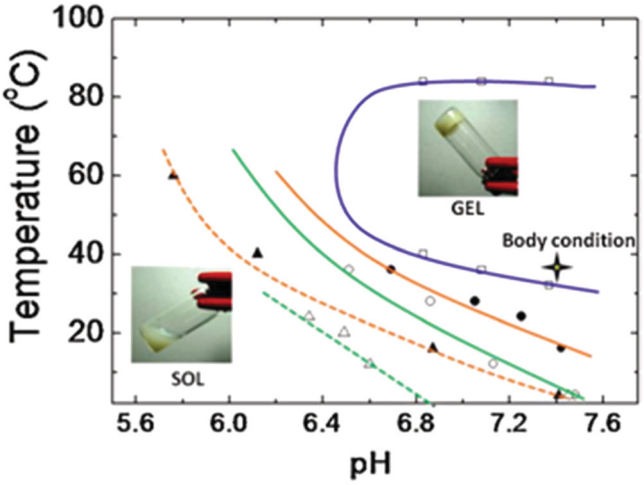

Figure 5. Phase diagrams of (□) 25 wt% PAU solution, (●) 20 wt%, (○) 25 wt% pH/thermo-responsive poly(amino urethane) multiblock copolymer solutions, (▲) 20 wt%, and (△) 25 wt% pH/thermo-responsive poly(amino urethane) multiblock copolymer solutions. The coordinate values at physiological pH and temperature, i.e., pH 7.4 and 37 °C. The inset pictures are the representative illustrations of the flowing liquid and the solid gel in the sol state and the gel state, respectively. Reproduced with permission.[62] Copyright 2013 John Wiley & Sons.

3.1.3. Pain Relief

Postoperative pain, which follows musculoskeletal surgeries, requires clinical intervention for purposes of patient comfort. Repeatedly injecting therapeutics is not ideal for the treatment of musculoskeletal postoperative pain. While there are a variety of options, all of these methods are far from ideal. Using local anesthetics to induce analgesia is a promising approach, however, local anesthetics have short half-lives, cause local tissue site reactions, as well as systemic toxicity. Liposomes, microparticles, and nanoparticles have been used as potential carriers but the diffusion effect means that these systems are not ideal as an injectable solution. In a recent report, ropivacaine was encapsulated with dexamethasone within a chitosan-based thermogelling system for the enhancement of neural blockade (**Figure 6**).[85] This system was able to limit sensory function and motor function for 2 d as shown by a rat sciatic neural blockade model.

3.2. Tissue Engineering

Tissue engineering is defined as an "interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ."[86] Tissue engineering can be applied for the recovery of injured tissue by using cells obtained by three main methods. Cells can be obtained from the affected patient. Such autologous cells are generally compatible with the patient although the availability of the cells could be a limiting factor. Alternatively, allogeneic cells can be obtained from a donor. Finally, when cells are obtained from other species, these cells are known as xenogenic cells. These cells should ideally be delivered to the injury site in the simplest non-invasive method, have its viability preserved during the administration and be allowed to proliferate at the injury site. As a result, the design of the synthetic carriers of cellular material is an important factor to consider. In order to fine-tune the material behavior towards cells, explicit functionalities have to be incorporated into materials to enhance its behavior towards cells. This requires understanding of cells, factors, extracellular matrices, and intercellular communications. The idea of using hydrogels for tissue regeneration has been summarized by a recent review.[87] Cells are conventionally cultured on a 2D cell culture plate, which is inherently different from a natural 3D environment in living systems. This results in differing cell morphologies, different expression of genes, and cytokine production. Hydrogels, with its interstitial space can be made up by as much as 90% (w/w) water. Ideally, thermogels undergo reversible sol–gel transitions upon exposure to physiological temperature without the need for chemical crosslinkers for gel formation and forms materials with similar mechanical properties as living tissues. The 3D network should allow for cell migration, attachment as well as diffusion of physiological fluids. The materials that make up the thermogel should be biocompatible and provide a suitable environment for cell proliferation and differentiation. Ideally, it should also be biodegradable and its degradation products should be

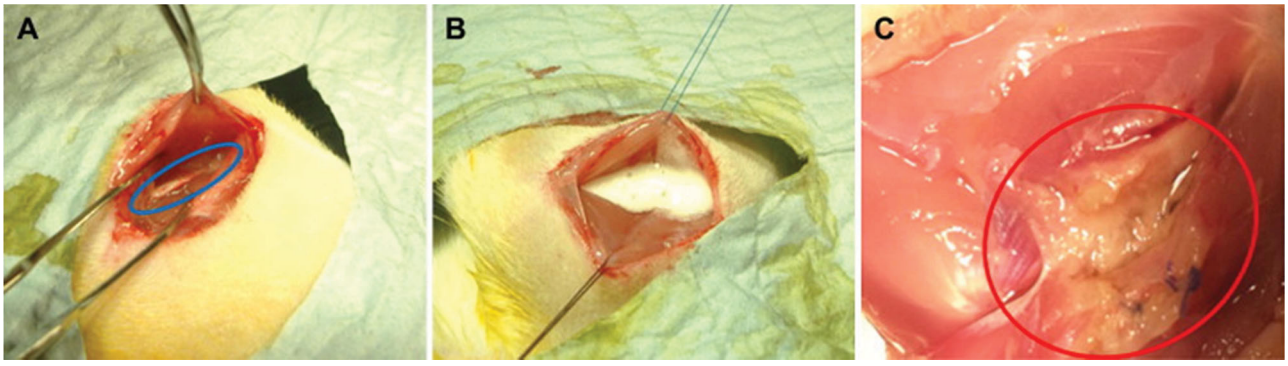

Figure 6. A) The biceps femoralis muscle of the right hind limb was opened and the common sciatic nerve was exposed (blue ellipsoid). B) The sciatic nerve was coated with ropivacaine/dexamethasone-loaded chitosan thermogel. C) Upon necropsy 7 d after surgery, the chitosan thermogel is still surrounding the sciatic nerve (red ellipsoid). Reproduced with permission.[85] Copyright 2013, Elsevier.

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compatible with the host tissue. Thermogels, which meet these criteria, have good potential as delivery vehicles for therapeutic agents and serve as cell culture scaffolds and allows the successful regeneration of tissue. In order to function well as a cell depot, a thermogel needs to have suitable mechanical strength and allow for the proliferation of cells within its matrix.

3.2.1. Cell Encapsulation

Choi et al. developed a thermogelling blend comprising glycidyl methacrylated chitooligosaccharide (COS) and diacrylated Pluronic F127.[88] A basic fibroblast growth factor (bFGF) and heparin complex was incorporated into the gel matrix to enhance cell proliferation within the gel. Upon irradiation with UV light, the thermogel sets to form an insoluble chemical gel. The elastic modulus and the erosion rate of the gel can be adjusted by changing the composition of the blend that showing the chemically cross-linked COS/Pluronic hydrogel is useful for a protein-delivery system and tissue-regeneration scaffold. Nagahama et al. reported a thermally responsive polymer as injectable cellular scaffold in 2008.[64] The partially cholesterol-substituted eight-arm poly(ethylene glycol)-block-poly(L-lactide) (8-arm PEG-*b*-PLLA-cholesterol) exhibited immediate gelation at 34 °C when the concentration of the polymer was above 3 wt%. The potential of the application of the polymer was examined by monitoring the viability and proliferation of L929 cells, which were cultured in a matrix fabricated with an extracellular matrix (ECM)-like micrometer-scale network structure inside the hydrogel. They observed a gradual and complete erosion of the thermogels over a month. Cho et al. developed an injectable thermosensitive water-soluble chitosan-g-PNIPAAm gel and studied the ability of the gel to encapsulate mesenchymal stem cells (MSCs) and differentiate to chondrocytes.[89] An in vivo study was performed to assess cartilage formation in the submucosal layer of the bladder of rabbits and good cartilage formation was observed in the bladder tissue. In another study, thermogelling polymers based on a blend of hydroxyethyl cellulose (HEC) and chitosan-glycerophosphate (CH-GP) were prepared.[90] Mouse bone mesenchymal stem cells (BMSC) were cultured on 2D films and inside 3D gels and showed good biocompatibility. Chondrocytes were cultured in Pluronic-g-chitosan thermogel.[91] Good proliferation of cells and increased glycosaminoglycan was produced after a month of culture. Pluronic thermogels appended with polyalanine were used for the encapsulation of chondrocytes.[92,93] Chondrocytes cultured in standard cell culture plate and Matrigel (3D control) exhibited a fibrous morphology after a month. When the chondrocytes were cultured in these thermogels, the spherical phenotype was maintained and expressed significantly greater amounts of sulfated glycosaminoglycan and type II collagen. Controllable gel modulus and gel nanostructure was demonstrated for these thermogels. These thermogel provided a friendly microenvironment at an initial polymer concentration of 10 wt% for the 3D culture of chondrocytes. Jeong et al. studied the possibility of using thermogels as an artificial matrix where the various processes of wound healing occurs by the processes going on in the encapsulated fibroblasts in the gel (**Figure 7**).[94] The 3D culture of fibroblasts in vitro was studied and compared with the commercially available Matrigel. The thermogel was comparable with Matrigel in terms of cell proliferation and performed much better than Matrigel for collagen types I and III formation. Wound healing was also studied in vivo where thermogel lacking encapsulated fibroblasts and phosphate-buffered saline (PBS) were used for control experiments to show the implication of the cell therapy with the thermogel. The thermogel with encapsulated fibroblast accelerated wound closure and improved epithelialization and the formation of skin appendages compared with the controls.

3.2.2. Cardiac Applications

The injection of materials into the ventricular wall as a bulking agent has been proposed as a method to prevent progressive adverse remodeling after a heart attack. A thermogelling copolymer based on *N*-isopropylacrylamide (NIPAAm), acrylic acid (AAc), and hydroxyethyl methacrylate-poly(trimethylene carbonate) (HEMAPTMC) was synthesized for this purpose.[95] It forms a semi-solid gel at 37 °C, and eroded over a 5 month period in vitro through hydrolytic cleavage of the PTMC residues. The infarcted left ventricular (LV) wall of a rat was injected with the thermogel or PBS as a control. In the control group, LV cavity area increased and contractility decreased after 8 weeks, while the thermogel group showed a preservation of both parameters during this period. Tissue ingrowth was observed in the thermogel injected area and a thicker LV wall and higher capillary density were found for the hydrogel versus PBS group. This material potentially offers an attractive biomaterial-centered treatment option for ischemic cardiomyopathy. Cell implantation has been suggested as a method to improve cardiac function after myocardial infarction. However, the poor engraftment and low survival rates of the transplanted cells within the ischemic tissue means that this therapy has not been widely adopted. The implantation of bone-marrow-derived mononuclear cells (BMMNCs) encapsulated in a Dex-PCL-HEMA/PNIPAAm thermogel increases cell engraftment and helps to restore cardiac function of rabbits, which had myocardial infarction induced.[96] A month after treatment, echocardiographic studies showed that this therapy preserved LV expulsion fraction and reduced LV dilatation. Neovascular formation was observed and scar expansion was prevented compared with the control experiments. Stem cell treatment for myocardial infarction has seen a promising new dawn based on recent animal studies and clinical trials. However, as a result of particularly poor cell viability and cardiac differentiation in infarcted hearts, it is still impractical for general clinical application. It has been reported that less than 2% of transplanted cells survived after 2 weeks, and even fewer differentiated into cardiac cells for rejuvenation of new heart tissue. In a recent report, Li et al. has shown that a PNIPAAm-based thermogel can induce the differentiation of MSCs into cardiomyocyte-like cells at high efficiency when the hydrogel matches the stiffness of the heart muscle of about 45 kPa.[97] Cell survival was enhanced by the inclusion of basic fibroblast growth factor (bFGF) into the hydrogel. Even under the low oxygen conditions (1% O_2 and 1% FBS), bFGF enhanced MSC survival and differentiation in the hydrogel. The contrasting behavior can be observed after 2 weeks where 70.5% of

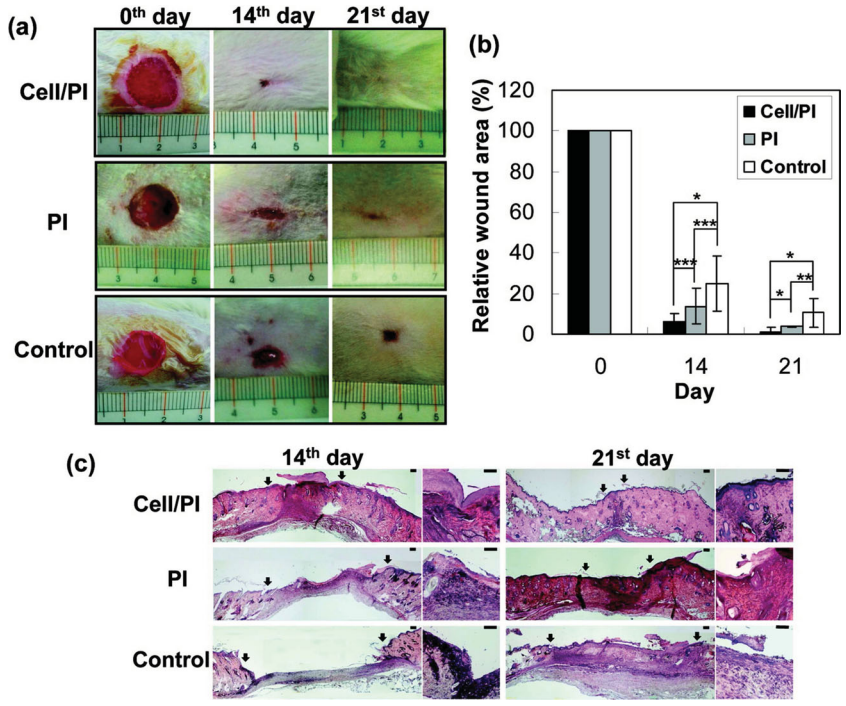

Figure 7. a) Photo images of wounds during the healing process. Cell encapsulating PEG-l-PA thermogel (cell/PI), cell-free PEG-l-PA thermogel (PI), and cell-free phosphate buffered saline (control) were compared on days 0, 14, and 21 after injury. b) Healing rate presented by relative wound area measured on days 0, 14, and 21 postinjury. A 100% indicates the wound area on day 0. $*p < 0.05$; $**p < 0.10$; $***p < 0.20$. c) Histology around the wounds compared on days 14 and 21 postinjury. The space between the arrows indicates the center of the original wound where the healing process is undergoing. The scale bar is 200 μm. The scale bar in the enlarged image is 100 μm. Reproduced with permission.[94] Copyright 2012, American Chemical Society.

MSCs in the bFGF-loaded hydrogel survived, while only 4.9% of MSCs remained in the hydrogel without bFGF. The bFGF-loaded thermogel is able to induce the differentiation of the cardiac cells. This was confirmed by the expression of genetic cardiac markers (MEF2C and CACNA1c) and proteins related to cardiac differentiation (cTnI and connexin 43). bFGF loading in the thermogels increased the paracrine effect of MSCs while also significantly increasing VEGF expression. Another similar work for cell encapsulation was demonstrated by the same group.[39] Here, flexible thermogels with mechanical properties similar to that of the rat and human myocardium can be made to respond synchronically with heart motion. Cardiosphere-derived cells (CDCs) were seeded in the thermogels and cultured for 2 weeks. The cells maintained their colony formation capability in the gel matrix. These thermogels could direct the differentiation of the CDCs to form mature cardiac cells. The mature cardiac-specific transcript factors cardiac troponin T (cTnT) and cardiac myosin heavy chain (MYH6) were up-regulated and the pre-mature cardiac marker GATA4 was down-regulated after 1 d of encapsulation. The differentiation of the CDCs was related to thermogel rigidity and amount of collagen in the thermogel. These thermogels possess physical properties suitable for myocardial injection and promotes CDC proliferation and cardiac differentiation, representing high-potential carrier candidates to deliver CDCs into hearts which are infarcted.

3.2.3. Bone Healing Applications

Nair et al. evaluated the in vivo biocompatibility of injectable thermogelling chitosan-AHP and the delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) in a bioactive form for bone healing applications.[98] The bioactivity of the released protein was retained based on the increase in alkaline phosphatase activity of mouse pre-osteoblast cells (MC3T3-E1). Ectopic bone formation was noted by histological and micro-computed tomography (μCT) evaluation with injection of a 4 μg mL^{-1} rhBMP-2-loaded chitosan-AHP gel. Lee et al. developed a pH- and thermo-sensitive polymer scaffold for autologous bone tissue regeneration by adding pH-sensitive sulfamethazine oligomers (SMOs) to both ends of a thermo-sensitive poly(ε-caprolactone-*co*-lactide)-poly(ethylene glycol)–poly(ε-caprolactone-*co*-lactide) (PCLA-PEG-PCLA) block copolymer.[68] The copolymer solution could rapidly and reversibly transform from a stable gel at physiological conditions (pH 7.4 and 37 °C) to a sol at pH 8.0 and 37 °C. It was observed that human mesenchymal stem cells (hMSCs) and recombinant human bone morphogenetic protein-2 (rhBMP-2) achieved encapsulation efficiencies of about 90% under physiological conditions. The potential of this polymer as an injectable scaffold for bone tissue regeneration was demonstrated when the integrity of the gel as well as hMSC differentiation was observed 7 weeks after injecting a polymer solution containing hMSCs and rhBMP-2 into mice (**Figure 8**). In another example, a biocompatible injectable thermogel composite made of hyaluronic acid-g-chitosan-g-PNIPAAm and embedded biphasic calcium phosphate microparticles was used to replace mineralized matrix.[99] The purpose of the gel matrix was to provide osteoblast cells with a 3D environment that is similar to that of a bone matrix. Human fetal osteoblast cells were found to be viable, had good proliferation characteristics, and maintained their osteoblastic differential potentials when cultured the thermogels. It was found that embedding the calcium phosphate enhanced the performance of the thermogel as an injectable carrier for bone cells. When the thermogel composites were implanted in nude mice through a subcutaneous injection, ectopic bone tissue was formed from the injected cell mass formed in the gel matrix.

3.2.4. Eye Treatment

As thermogelling polymers can be fabricated as transparent materials, they can find applications in eye treatment or dioptric applications. Hydrogels have been fabricated into contact lenses and been extensively studied.[100,101] Duwuri et al. reports a formulation of ganciclovir (GCV)-loaded PLGA microspheres dispersed in thermogelling PLGA-PEG-PLGA gel for

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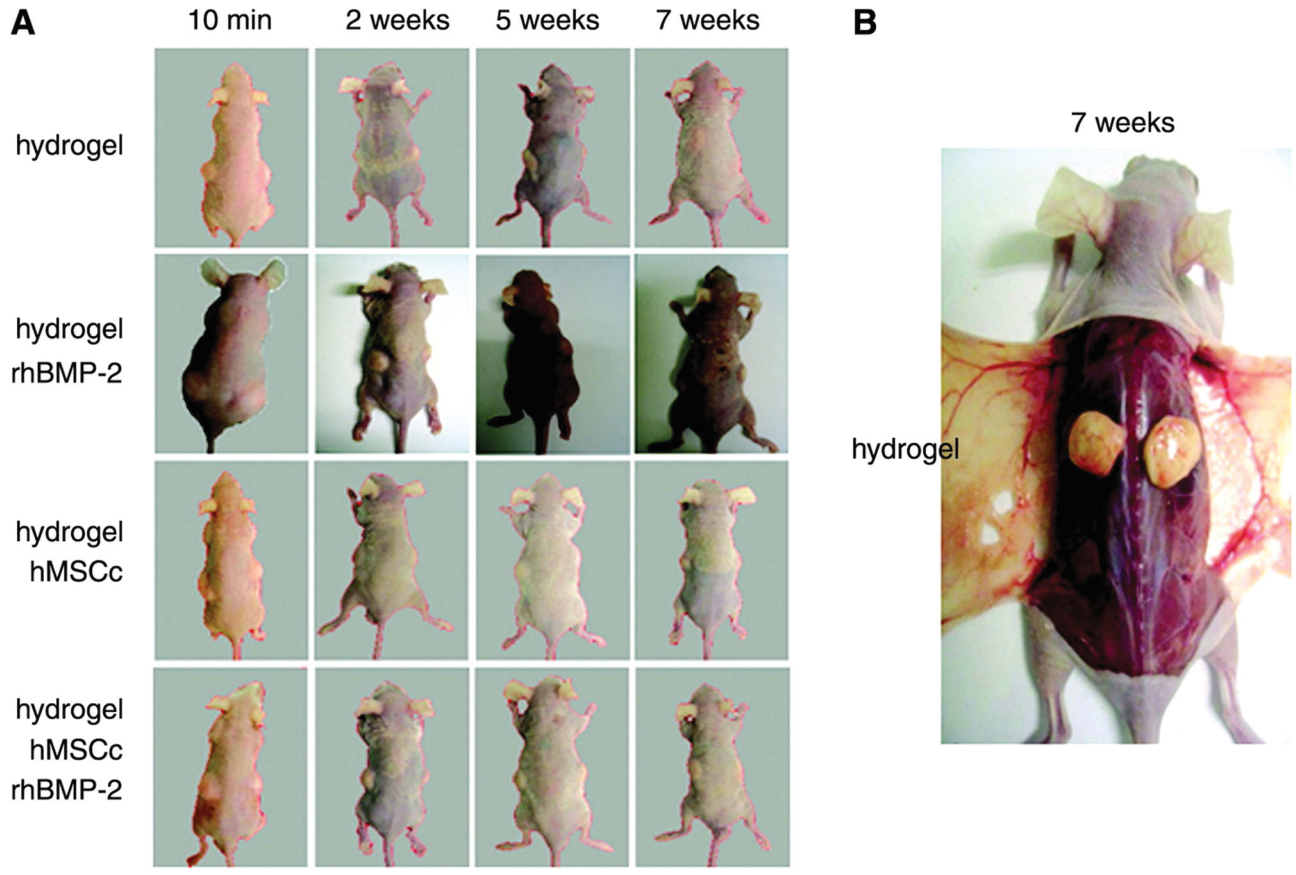

Figure 8. A) Nude mice following subcutaneous injection of the pH/thermo-sensitive block copolymer alone, with rhBMP-2, with hMSCs, or with hMSCs and rhBMP-2. B) The pH/thermo-sensitive hydrogel maintained its original morphological characteristics for 7 weeks. C) Macroscopic observations of hydrogels at 7 weeks postinjection, after the hydrogel samples had been removed from the injected sites. Reproduced with permission.[68] Copyright 2009, Mary Ann Liebert.

intravitreal delivery.[102] A constant in vitro GCV release profiles was obtained from the formulation. The amounts of GCV entrapped in the microspheres were sufficient to administer therapeutically relevant doses in 60 µL of the formulation. A direct vitreous injection of GCV resulted in the maintenance of concentrations in the vitreous for about 2.5 d, whereas the gel formulation produced steady-state GCV levels in the vitreous for approximately 14 d. Wang et al. recently reported the sustained release of bevacizumab from a triblock thermogelling copolymer of poly(2-ethyl-2-oxazoline)-*b*-poly(ε-caprolactone)-*b*-poly(2-ethyl-2-oxazoline) (PEOz-PCL-PEOz).[103] Bevacizumab is an antibody used clinically to treat intraocular neovascular ailments based on its antivascular endothelial growth factor (VEGF) character. However, this therapy is limited by the short half-life of bevacizumab and thus requires a carrier, which protects the bioactivity of bevacizumab as well as provides a sustained release of the drug. The PEOz-PCL-PEOz thermogel has good potential and showed no significant cytotoxicity to human retinal pigment epithelial cells. A toxicity study was carried out to compare the PEOz-PCL-PEOz thermogel with Matrigel and Pluronic F127.[104] Both Matrigel and Pluronic F127 show lens and neuroretinal toxicities. On the other hand, PEOz-PCL-PEOz thermogel does not show detectable ocular tissue toxicity. The morphology and electrophysiology of the retina were well preserved after 2 months of intravitreal injection of the PEOz-PCL-PEOz thermogel. Misra et al. demonstrated the injection of P(NIPAAm-co-Dex-lactate-HEMA) thermogels into the subconjunctival space of rat eyes for sustained release of insulin to the retina.[105] Moreover, thermogelling polymers were also designed for ocular treatment. In another report, a reverse thermogelling polymer, poly(ethylene glycol)-poly(serinol hexamethylene urethane) (ESHU) was developed for the treatment of age-related macular degeneration (AMD).[106] Bevacizumab release from the ESHU gels was studied and the release was sustainable over 17 weeks in vitro. The release kinetics of the drug from the gel was affected by drug dose and ESHU concentration. The results show that ESHU could be potentially used for ocular drug delivery. Thermogelling PNIPAAm/hyaluronic acid copolymers were developed as cell delivery vehicles for application as injectable retinal therapeutics.[58] These novel copolymers were designed to deliver a liquid suspension of cells in an injectable manner into the subretinal space for treatment of retinal degenerative ailments such as AMD and diabetic retinopathy. The transplanted cells were encapsulated within the gel matrix by utilizing the thermogelling effect. These cell-adhesive thermogels demonstrated very good compatibility with retinal pigment epithelial (RPE) cells in culture and negligible host response was detected upon subcutaneous embedding of the gel depot in vivo.

3.2.5. Neural Regeneration Treatment

The repair of injured neural conduits in the central nervous system requires innovative neural tissue regeneration strategies. Optimum neuron and neurite interactions with the scaffold material are essential and needs to be controlled. Nisbet el al. studied the applicability of using xyloglucan-based and xyloglucan-graft-poly-D-lysine (PDL) thermogelling polymers for neural tissue regeneration applications.[107] The thermogels were injected into the caudate putamen of adult rats. Axon infiltration in a controlled manner was observed. The microglia reaction to the thermogels peaked after 3 d before subsiding to homeostatic levels after 28 d. The microglia did not penetrate into the scaffold with the cells largely accumulating at the scaffold–tissue interface. The peak activation of astrocytes occurred between 14 and 21 d for these thermogels. Thermogels having higher concentrations of grafted PDL demonstrated enhanced infiltration levels for astrocytes and neurites. It was found that the migration of the astrocyte migration happened concurrently with neurite infiltration within the scaffolds. This suggests that astrocytes, through the secretion of laminin, may have enabled this infiltration.

3.2.6. Prevention of Tissue Adhesion

Tissue adhesions typically form after abdominal surgery. Although improved surgical techniques, such as the reduction of surgical trauma, prevention of ischemia, and avoidance of exposure of the peritoneal cavity to foreign materials, reduce tissue adhesion, this is not completely eliminated. Biodegradable thermogelling polymers have been utilized to prevent the post-operative tissue adhesion. A biodegradable triblock copolymer poly(ε-caprolactone-*co*-lactide)-b-poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-lactide) (PCLA-PEG-PCLA) was synthesized.[108] This polymer showed low cytotoxicity and did not induce hemolysis. For in vivo evaluations, a rabbit model of sidewall defect-bowel abrasion was employed. Postoperative peritoneal adhesion was significantly reduced when PCLA-PEG-PCLA thermogels was applied on the wound site. PCL-PEG-PCL thermogels have also been reported to prevent postoperative adhesions.[109] In vivo studies revealed that the animals which had the administered thermogels did not develop adhesions whereas the untreated animals developed adhesions that could only be separated by sharp dissection. The thermogel adheres to peritoneal wounds and degraded gradually over about a week and resorbed into the body. Ding et al. reported recently that free RGD peptides can help to prevent the adhesion of tissue by hindering focal adhesion between cells and surfaces of barrier devices.[110] This idea was based on two concepts, firstly, the thermogel acts as a physical barrier to prevent adhesion between injured abdominal wall and secondly, the RGD molecules act as integrin blockers upon release from the thermogel to prevent tissue adhesion. PCLA-PEG-PCLA thermogels were used to encapsulate cyclic peptides cyclo(-RGDFK-). The rabbit model of sidewall defect and bowel abrasion was used to study the in vivo anti-adhesion efficacy. The PCLA-PEG-PCLA thermogels with the encapsulated peptides significantly reduced postoperative peritoneal adhesion.

4. Conclusion

This Review covered several hot biomedical and clinical applications of thermogelling polymers in recent years. In the first part, we have summarized some methods for the synthesis of thermogelling polymers. In the second part, biomedical applications such as drug delivery and tissue regeneration applications were comparatively discussed with specific examples highlighted to demonstrate the applications. With the advent of new polymeric materials, more thermogelling polymers may be developed on the top of those currently available, which could expand the scope of applications in the biomedical field. The thermogelling polymer can be designed with extra sensitivity to environmental stimuli for improved assay sensitivity or treatment efficacy. With the great promise provided by these materials, the issues regarding long-term safety, degradation and clearance pathway should be addressed as well. Biocompatibility of a material with drug as well as the host at the implanted site should be confirmed. A typical mild inflammation of the thermogel implant accompanies collagen capsule formation, followed by degradation of the polymer and the surrounding collagen capsule. If the collagen capsule is thick, it can affect the drug release kinetics. In addition, the drug should be compatible with the polymer and the degradation products of the polymer. For example, the degradation of polyesters leads to the production of carboxylic acids. The resultant lower pH might denature the encapsulated proteins or cells. Formulation of the thermogelling polymers with proteins and drugs is also a critical factor as phase separation might occur during the course of the drug release. In using thermogels for 3D cell culture and tissue regeneration applications, a complete understanding of the biological activities of the cells as well as aspects of physiology is critical. For cells requiring anchoring sites in the thermogel, peptides, sugars, and other receptor–substrate interaction moieties which operate at the cellular level can be incorporated in the design of the cell culture matrix. The nanostructure of thermogel can also be made to resemble biological extracellular matrix, which has well-defined nanofibers of collagen dispersed in the hyaluronic acid and proteoglycan. Furthermore by utilizing the thermogel, genetically modified cells, such as growth hormone secreting cells can be made to function in an isolated environment. We believe that thermogelling polymer can be exploited for more rational applications in the near future. The progress of thermogelling polymers has vast potential in the landscape of biomedical applications. The further development of thermogelling polymers is an area of research, which will be closely watched in the future.

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