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Biodegradable Thermogels

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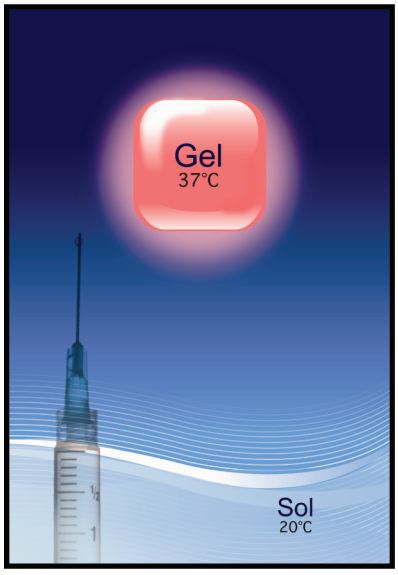
CONSPECTUS

All living creatures respond to external stimuli. Similarly, some polymers undergo conformational changes in response to changes in temperature, pH, magnetic field, electrical field, or the wavelength of light. In one type of stimuli-responsive polymer, thermogel polymers, the polymer aqueous solution undergoes sol-to-gel transition as the temperature increases. Drugs or cells can be mixed into the polymer aqueous solution when it is in its lower viscosity solution state. After injection of the solution into a target site, heating prompts the formation of a hydrogel depot in situ, which can then act as a drug releasing system or a cell growing matrix.

In this Account, we describe key materials developed in our laboratory for the construction of biodegradable thermogels. We particularly emphasize recently developed polypeptide-based materials where the secondary structure and nanoassembly play an important role in the determining the material properties. This Account will provide insights for controlling parameters, such as the sol−gel transition temperature, gel modulus, critical gel concentration, and degradability of the polymer, when designing a new thermogel system for a specific biomedical application.



By varying the stereochemistry of amino acids in polypeptides, the molecular weight of hydrophobic/hydrophilic blocks, the composition of the polypeptides, the hydrophobic end-capping of the polypeptides, and the microsequences of a block copolymer, we have controlled the thermosensitivity and nanoassembly patterns of the polymers. We have investigated a series of thermogel biodegradable polymers. Polymers such as poly(lactic acid-*co*-glycolic acid), polycaprolactone, poly(trimethylene carbonate), polycyanoacrylate, sebacic ester, polypeptide were used as hydrophobic blocks, and poly(ethylene glycol) and poly(vinyl pyrrolidone) were used as hydrophilic blocks. To prepare a polymer sensitive to pH and temperature, carboxylic acid or amine groups were introduced along the polymer backbone. The sol−gel transition mechanism involves changes in the secondary structures of the hydrophobic polypeptide and in the conformation of the hydrophilic block. The polypeptide copolymers were stable in the phosphate buffered saline, but the presence of proteolytic enzymes such as elastase, cathepsin B, cathepsin C, and matrix metalloproteinase accelerated their degradation.

We also describe several biomedical applications of biogradable thermogel polymers. One subcutaneous injection of the insulin formulation of thermogel polypeptide copolymers in diabetic rats provided hypoglycemic efficacy for more than 16 days. The thermogels also provided a compatible microenvironment for chondrocytes, and these cells produced biomarkers for articular cartilage such as sulfated glucoaminoglycan (sGAG) and type II collagen. The thermogels were also used as a fixing agent for in situ cell imaging, and cellular activities such as endocytosis were observed by live cell microscopy.

Introduction

Our body is actually a hydrogel in equilibrium with water. A thermogel is an aqueous polymer system that undergoes solution (sol)-to-gel transition as the temperature increases. Thermogels have been highlighted during the past decade as a minimally invasive implantable system.[1−3] Since the pioneering research on biodegradable thermogels using poly(lactic acid-*co*-glycolic acid)/poly(ethylene glycol)

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(PLGA/PEG),[4−7] chitosan/glycerol phosphate,[8] polyphosphazene,[9−11] polycaprolactone,[12−16] polycarbonate,[17] multiblock poly(ethylene glycol)/poly(propylene glycol) (PEG/PPG),[18−20] polycyanoacrylate,[21] polyorthoester,[22] poly(*N*-(2-hydroxyethyl) methacrylamide-lactate),[23] poly(propylene phosphate),[24] and polypeptides[25−31] have been reported. By introducing multiple sensitivities to the characteristics of a thermogel, pH/temperature sensitive sol−gel transition polymer systems were also reported.[32−36] Clinically, the most advanced thermogel is the PLGA/PEG system (OncoGel), which is a sustained release formulation of paclitaxel for use in treatment of brain cancer, esophageal cancer, and breast cancer, and is in phase II clinical trials being conducted by Protherics.[37]

After examining the problems associated with Pluronics as a long-term drug delivery vehicle, such as short gel duration (<1 day) after implanting the hydrogel in the subcutaneous layer, we have developed a series of biodegradable thermogelling systems to address the following issues.

(1) How to design a durable and degradable thermogel.
(2) How to increase the spectrum of thermogel duration from a week to several months.
(3) How to shorten the reconstitution time of the thermogel formulation.
(4) How to design a disease-responsive drug releasing thermogel.
(5) How to design a biocompatible and storageable thermogel which begins to degrade after practical in vivo application.

In this Account, we describe our rationale used for designing a thermogelling material to address the above issues during the past decade. In particular, recently developed polypeptide thermogels are emphasized and the future perspectives of using the thermogel are suggested.

How to Design Pluronics Alternatives with Durability and Degradability

Pluronics or poloxamers, triblock copolymers of PEG-PPG-PEG, aqueous solutions in a certain concentration range undergo temperature-sensitive sol-to-gel transition as the temperature increases.[38] When the aqueous polymer solution is injected into the subcutaneous layer of a warm-blooded animal, a hydrogel is formed by temperature-sensitive sol-to-gel transition. However, the formed gel does not persist for more than 1 day and, therefore, has been applied as a short-term implantable system for

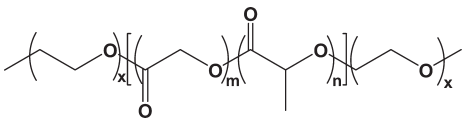

FIGURE 1. Structure of PEG-PLGA-PEG.

pharmaceutical agents or formulation excipients to solubilize hydrophobic drugs.[5,39] In addition, the repeating units of Pluronics consist of ether functional groups which resist in vivo degradation. To address these problems, PEG-PLGA-PEG was designed (Figure 1).[4,6] The rationale for this structure can be summarized as follows. First, PLGA has hydrolyzable ester linkages, which are degraded in the subcutaneous layer of a mammal. Second, hydrophobicity/hydrophilicity of the polymer can be controlled not only by a ratio of the molecular weight of PLGA to that of PEG but also by the ratio of lactic acid to glycolic acid of PLGA. These molecular parameters also affect the degradation kinetics and gel modulus. Third, intermolecular interactions among PEG-PLGA-PEG are stronger than those for Pluronics; therefore, the short gel duration problem experienced with Pluronics is to be improved. Fourth, the final degradation products of the PEG-PLGA-PEG are lactic acid, glycolic acid, and PEG, which are approved as safe materials by the United States Food and Drug Administration.[40]

A thermogelling PEG-PLGA-PEG was developed through studies on structure−property relationships by varying the molecular weights of PEG and PLGA.[4,40] When the PEG molecular weight was >2000 Da, the PEG-PLGA-PEG aqueous solutions showed gel-to-sol transition as the temperature increased. However, as the molecular weight of PEG decreased to <1000 Da, a very durable thermogelling system of PEG-PLGA-PEG that showed sol-to-gel transition as the temperature increased was formed. The phase diagram of PEG-PLGA-PEG could be controlled by varying the molecular weights of PEG and PLGA, and the ratio of lactic acid to glycolic acid.[4] The difference in gel duration of PEG-PPG-PEG (Pluronics) and PEG-PLGA-PEG originates from the difference in molecular-assembly when forming a gel. Pluronics forms a gel by packing the micelles, whereas PEG-PLGA-PEG forms a gel by increases in molecular attraction and micellar aggregation accompanying significant percolation among the crew-cut micelles with short chain PEGs.[4,41,42] In addition, the PEG-PLGA-PEG becomes more hydrophobic during degradation, by preferential mass loss of the PEG-rich segment of the degradation product.[5,43] The decrease in molecular weight of PEG-PLGA-PEG (550−2810−550) from 4200 to 2600 Da and a mass loss of ∼30% were observed

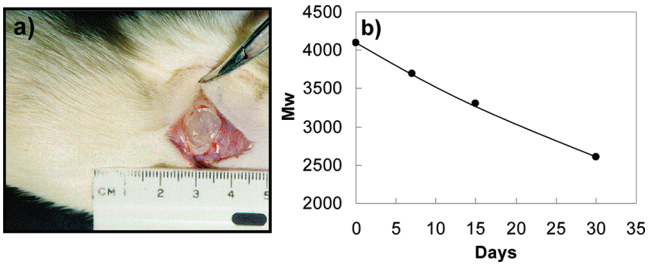

FIGURE 2. (a) Gel formed in the subcutaneous layer of a rat by injecting the PEG-PLGA-PEG aqueous solution. (b) Change in molecular weight of the polymer in the subcutaneous layer of rats. Adapted from ref 5 with permission. Copyright 2000 Wiley-VCH.

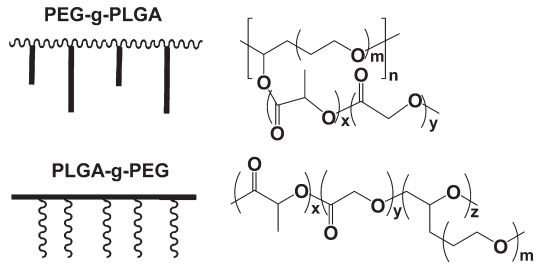

FIGURE 3. Structures of PEG-*g*-PLGA and PLGA-*g*-PEG.

during a 1 month incubation in rats (Figure 2).[7] A 2 month release profile for a hydrophobic model drug (spironolactone) was observed by using the PEG-PLGA-PEG (550−2810−550) thermogel.[6] To conclude, the short gel duration as well as biodegradation problems of Pluronics were solved by PEG-PLGA-PEG, where the PPG of Pluronics was replaced by PLGA.

How to Broaden the Duration Spectrum of PLGA Thermogel

By adjusting the triblock topology of PEG and PLGA, that is, PEG-PLGA-PEG or PLGA-PEG-PLGA, the duration of the thermogel could be varied over a time span of 1−2 months.[5−7] However, we need to broaden the gel duration over 1 week to several months, because some systems need a 1 week delivery system, while others need >2 months. Two graft copolymer systems of PEG-*g*-PLGA and PLGA-*g*-PEG were designed (Figure 3). In the case of PEG-*g*-PLGA, the degradation of the pendant PLGA made the remaining PEG-*g*-PLGA more hydrophilic, and the polymer became soluble in water as a sol at 37 °C. On the other hand, the degradation occurred along the backbone of PLGA in PLGA-*g*-PEG. The remaining polymer became more hydrophobic by the preferential mass loss of the PEG-rich moieties, which prolonged the duration of the hydrogel. The thermogel of PEG-*g*-PLGA showed a 1 week duration as a gel, whereas the PLGA-*g*-PEG showed a 3 month duration as a gel, even though both polymer aqueous solutions of PEG-*g*-PLGA and PLGA-*g*-PEG showed similar phase diagrams.[44−47]

How to Shorten the Dissolution Time

The development of a biodegradable and durable system of PEG/PLGA became a milestone in biodegradable thermogel research. Since then, several other thermogelling systems, such as chitosan/glycerol phosphate, poly(propylene fumarate), and polyphosphazenes, have been reported.[8,9,48] However, the polymers required a long dissolution time to prepare an aqueous solution. In the case of PEG/PLGA, it required 8 h to make a 1.0 mL aqueous solution. To solve the problem, a crystalline biodegradable polymer of polycaprolactone (PCL)-based thermogel was designed. The thermogelling polymer of PEG-PCL-PEG (550-2100-550) has a melting point of 40−50 °C (Figure 4).[12,13] Below the melting point of the polymer, it exists as a powder form. Above the melting temperature, the polymer/water exists as an oil/water liquid phase. After vortex-mixing the liquid phase, the mixture is quenched in an ice bath and then becomes a transparent aqueous solution within 1 min. Therefore, we can produce an aqueous polymer solution in 1 min, instead of the 8 h required for the previous systems. While the PEG/PCL systems have the advantage of rapid preparation of their aqueous solutions, they tend to form a physical gel by crystallization of PCL blocks when standing for more than 1 h.[14] Therefore, the system needs a heating and cooling cycle for reconstitution before the injection of the formulation. The crystallization of the PCL block is partially reduced by copolymerizing caprolactone with lactide or trimethylene carbonate.[14,15]

Pluronics Are Still Attractive Thermogels

Aside from the research on thermogelling polyesters of PLGA and PCL, multiblock copolymers of Pluronics have been designed to solve the short gel duration problem of Pluronics. The mutiblock Pluronics are connected by terephthalate, 2,5-dicarboxy terephthalate, and disulfide (Figure 5). The mechanism of sol-to-gel transition of the multiblock copolymers of Pluronics changes from a simple micellar packing model of Pluronics to a micellar aggregation and percolation model accompanied by intermicellar bridging of the polymers. In fact, the gel duration of P85-terephthalate multiblock copolymer increased to >15 days, whereas that of unmodified P85 was <1 day.[49] The 2,5-dicarboxy terephthalate connected multiblock Pluronics

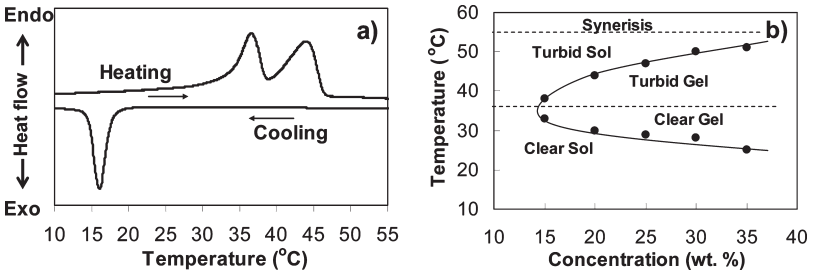

FIGURE 4. (a) Thermogram of PEG-PCL-PEG triblock copolymer. (b) Phase diagram of the polymer aqueous solution. Adapted with permission from ref 12. Copyright 2005 American Chemical Society.

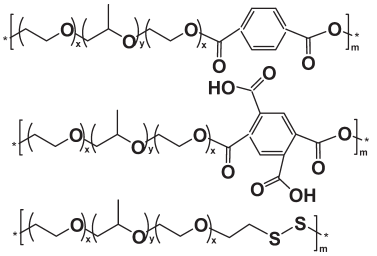

FIGURE 5. Structures of multiblock Pluronics derivatives.

aqueous solutions showed a gel state in a pH range of 4−7 and a sol state at low and high pHs.[35] The solubility of PEG in water increases at low pHs, whereas the degree of ionization of 2,5-dicarboxy terephthalate of the multiblock copolymer increases at high pHs. As another approach, random multiblock copolymers of PEG/PPG/biodegradable polyester, urethane connected PEG/PPG, and oligopeptide connected Pluronics have also been suggested by other groups as thermogelling polymers.[18−20,50,51] In particular, the thermogelling PEG/PPG/poly(hydroxy butyrate) system showed a very low critical gel concentration in water (<2.0 wt.%) due to the highly hydrophobic character of poly(hydroxy butyrate).[18]

As a biomolecule-sensitive degradable system, a multiblock Pluronics connected with disulfide bonds was designed. The disulfide bonds in the multiblock copolymer were degraded by glutathione. The concentration of glutathione increases around tumor tissue,[52] indicating that a disease-responsive drug delivery system can be designed by using this system. In phosphate buffered saline, there was no significant erosion of the multiblock P85 disulfide (P85-SS) thermogel for 12 days, whereas the degradation and drug release profiles of the P85-SS thermogel were significantly affected by the presence of glutathione (Figure 6).[53]

Stable in Vitro and Degradable in Vivo: Polypeptide-Based Thermogels

A thermogelling polymer that is stable in water, however degradable after biomedical application, was designed by the concept of an enzymatically degradable thermogel. As the first example, we designed a polypeptide-based thermogelling system. Polypeptides not only have unique secondary structures of α-helix, β-sheet, and random coil but also have a broad spectrum of hydrophobic and hydrophilic amino acids (Figure 7).[54] Therefore, they have provided abundant resources for creative biomaterial research.[55,56]

We designed thermogelling polypeptide copolymers by (1) controlling stereochemistry of polypeptides, (2) varying the molecular weights of hydrophilic and hydrophobic blocks, (3) varying the composition of polypeptides, (4) end-capping by hydrophobic alkyl groups, (5) controlling microsequences of polymers, and (6) introducing pH/temperature dual sensitivity.

PEG-L-PA (EG_{21}-L-A_{10}) and PEG-DL-PA (EG_{21}-DL-A_{11}) with similar block lengths were compared.[26] The PEG-L-PA with a β-sheet secondary structure formed a nanofibrous structure in water, and its aqueous solution (8.0 wt %) underwent thermogelation at 30 °C. On the other hand, thermogelation of PEG-DL-PA with a random coil secondary structure was observed at much higher temperatures (>60 °C) and concentrations (>16.0 wt %) than those of PEG-L-PA. As the PEG molecular weight varied 1000, 2000, and 5000 Da at a fixed L-PA molecular weight of 700 Da in PEG-L-PA, the secondary structure shifted from a β-sheet-rich polypeptide to a α-helix-rich polypeptide.[30] The conjugated PEG interrupts the packing of L-PAs into β-sheets by steric hindrance due to the highly dynamic nature of the PEG in water.[57] In addition, the resulting nanostructure changes from nanofibrous to micellar structures as the PEG molecular weight increases from 1000 to 2000 and to 5000 Da. When the molecular weight of PEG was fixed at 2000 Da and L-PA varied from 700 to 1500 Da, the β-sheet content decreased and the α-helix content increased, reflecting the intrinsically high tendency of L-PA to form an α-helix (Figure 8).

When the hydrophobic phenylalanines were incorporated in the polypeptide block, the sol-to-gel transition

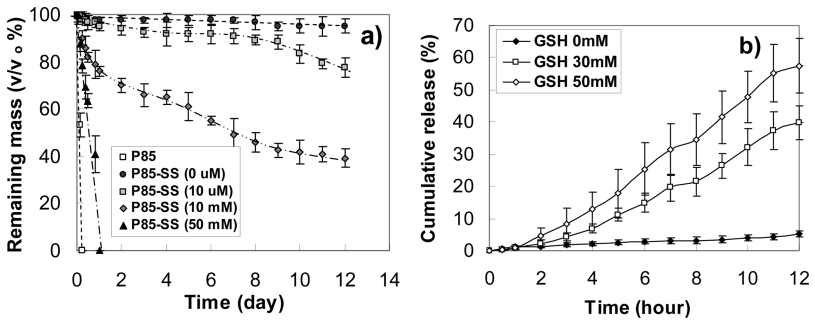

FIGURE 6. Effect of gluthathione (GSH) on the degradation (a) and paclitaxel release (b) from the Pluronics disulfide thermogel. Adapted with permission from ref 53. Copyright 2006 American Chemical Society.

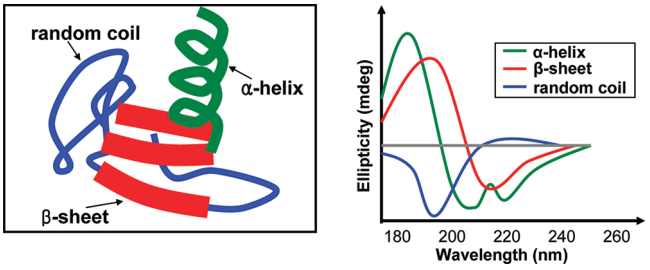

FIGURE 7. Typical secondary structures of polypeptides (left) and their characteristic circular dichroism (CD) spectra (right).

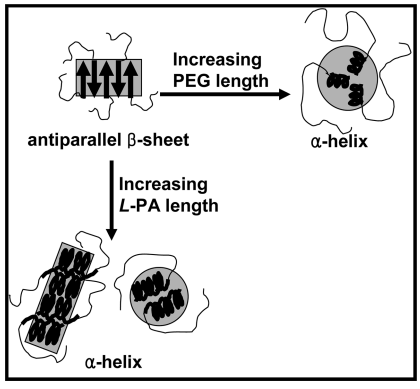

FIGURE 8. Effect of molecular weights of PEG-L-PA on the nanoassembly and secondary structures of polypeptides. Adapted from ref 30 with permission. Copyright 2010 RSC.

temperature and concentration significantly decreased. The sol-to-gel transition of PEG-PAF (EG_{20}-$A_{4.1}F_{1.0}$) aqueous solutions was observed at 8–22 °C in a concentration range of 3.0–7.0 wt % (Figure 9).[28]

The secondary structure of (DL/L)-poly(alanine)-poloxamers-(DL/L)-poly(alanine) ((DL/L)-PA-PLX-(DL/L)-PA) was particularly sensitive to the change in temperature. The β-sheet structure was strengthened as the temperature increased, which might play a role in driving the sol-to-gel transition of the (DL/L)-PA-PLX-(DL/L)-PA aqueous solution (Figure 10).[32] The end groups of (DL/L)-PA-PLX-(DL/L)-PA were modified by acetyl (C1), ethanoyl (C2), and propanoyl (C3) groups. As the hydrophobicity of the end-capping molecule increased from C0 (unmodified), C1, C2, and C3, not only the sol-to-gel transition temperature was lowered but also the β-sheet content and population of nanofibrous structures increased.[29,58] The hydrophobic alky groups act as a core for polymer assembly and thus facilitate the packing the PAs into β-sheets to form nanofibrous structures. This finding can be compared with the effect of hydrophilic PEG conjugated to PA, where the PEG interferes with the β-sheet structure formation of the PA.[30] Leucines were incorporated in the polypeptide to prepare the poly(leucine-*co*-alanine)-PLX-poly(leucine-*co*-alanine) (PAL-PLX-PAL). An order of Ala < Leu < Phe for effectiveness in lowering the sol-to-gel transition temperature and in increasing the β-sheet component of the polypeptide was observed, suggesting that increases in hydrophobicity and β-sheet content lower sol-to-gel transition temperature of the polymer aqueous solution.[59,60]

The effect of the polypeptide microsequences on the nanoassembly and thermosensitivity of a polymer aqueous solution was studied using triblock copolymers of poly(ethylene glycol)-DL-polyalanine-L-polyalanine (PEG-DL-PA-L-PA; EG_{44}-DL-A_9-L-A_9) and poly(ethylene glycol)-L-polyalanine-DL-polyalanine (PEG-L-PA-DL-PA; EG_{44}-L-A_9-DL-A_9).[61] The PEG-DL-PA-L-PA aqueous solution underwent a sol-to-gel-to-squeezed gel transition, whereas the PEG-L-PA-DL-PA aqueous solution underwent a sol-to-gel transition. CD spectra also showed the PEG-DL-PA-L-PA underwent α-helix-to-random coil transition at temperatures > 40 °C, whereas PEG-L-PA-DL-PA maintained its α-helical structure over a temperature range of 4–50 °C. PEG is in a highly dynamic state, and DL-PA with a random coil conformation is rather flexible, whereas L-PA has a rigid α-helical conformation in water. Therefore, PEG-DL-PA-L-PA has gradient flexibility along the polymer sequence in water. In addition, cryo-TEM images showed that the PEG-DL-PA-L-PA formed fibrous nanostructures, whereas

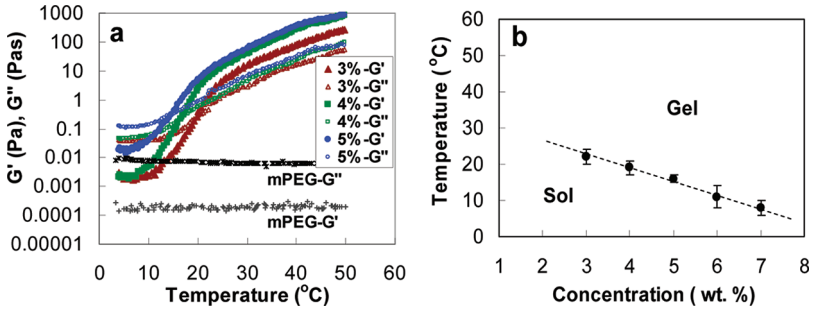

FIGURE 9. (a) Modulus of the PEG-PAF aqueous solutions as a function of temperature. (b) Phase diagram of PEG-PAF aqueous solutions. Adapted from ref 28 with permission. Copyright 2009 Elsevier.

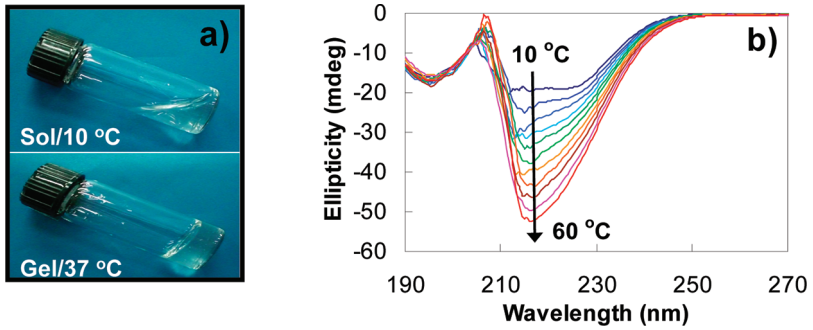

FIGURE 10. (a) Photos of sol (10 °C)/gel (37 °C) images. (b) CD spectra of (DL/L)-PA-PLX-(DL/L)-PA aqueous solution as a function of temperature. Adapted with permission from ref 27. Copyright 2008 American Chemical Society.

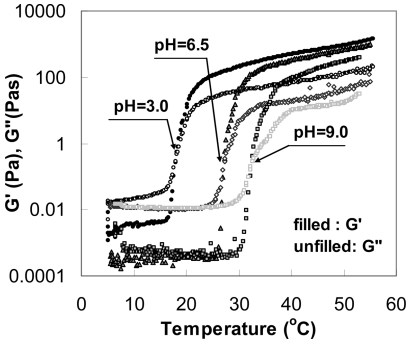

FIGURE 11. Changes in moduli of the CS-*g*-(PA-PEG) aqueous solutions (6.0 wt %) as a function of temperature and pH. Adapted from ref 34 with permission. Copyright 2011 RSC.

PEG-L-PA-DL-PA formed spherical micellar structures in water. The nanofibrous structure of PEG-DL-PA-L-PA with gradient flexibility along the polymer sequence might facilitate gel formation.

As an alternative to PEG, poly(vinyl pyrrolidone) (PVP) was used as a hydrophilic block of thermogelling polypeptides. With a similar molecular weight, the sol-to-gel transitions of PVP-PA were observed in a concentration range of 3.0−8.0 wt %, whereas those of PEG-PA were observed in a concentration range of 16.0−30.0 wt %, suggesting that PVP is more hydrophobic than PEG.[62]

To introduce a dual sensitivity of pH and temperature to the polymer aqueous solution, chitosan grafted with PEG-PA (CS-*g*-(PA-PEG)) was prepared.[34] The dehydration of PEG, deprotonation of chitosan, and strengthening of the secondary structure of the polypeptide were suggested as a mechanism for thermogelation of the CS-*g*-(PA-PEG) aqueous solution. The sol-to-gel transition temperature decreased as the pH decreased (Figure 11). Protonation of chitosan at an acidic pH of 3.0 creates extensive intermolecular hydrogen bonding among the polymers, whereas the CS-*g*-(PA-PEG) develops well-defined micellar structures at a basic pH of 9.0. Such differences contribute to the decrease in the sol-to-gel transition temperature of the CS-*g*-(PA-PEG) at the acidic pH. The phase behavior can be used for endoscopic injection therapy of stomach ulcers by forming a gel coating on the injured sites with acidic pH, while maintaining a low viscous sol state in the catheter at neutral pH.

The degradation of PA, PAF, and PAL was accelerated by proteolytic enzymes such as elastase, cathepsin B, cathepsin C, and matrix metalloproteinase-2 (MMP-2) in the subcutaneous layer of mammals.[28,60] Most protein drugs are administered by subcutaneous or intramuscular injection. In addition, the thermogelling polypeptide block copolymers

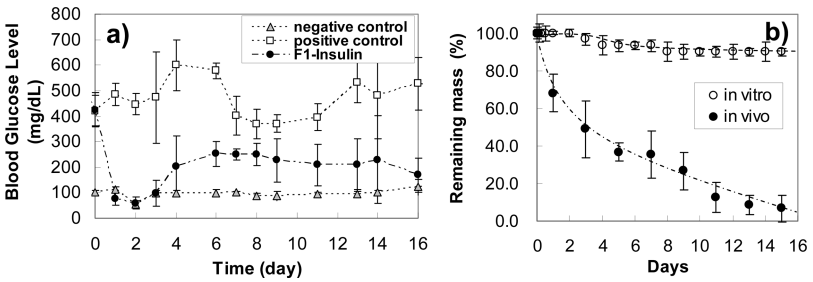

FIGURE 12. (a) Blood glucose level of the diabetic mice after a single subcutaneous injection of insulin/PEG-PAF aqueous solution (F1). (b) In vitro and in vivo degradation of the gel. Adapted from ref 28 with permission. Copyright 2009 Elsevier.

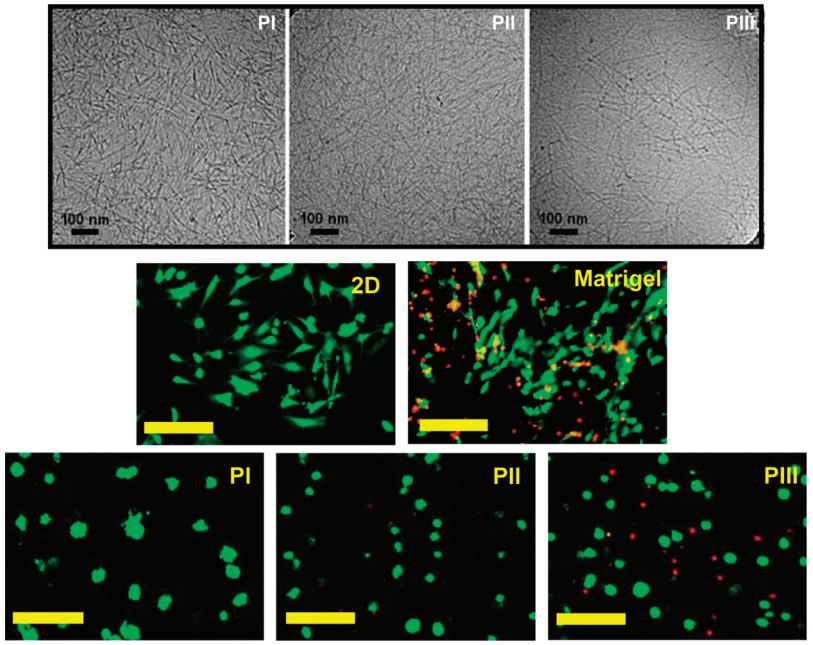

FIGURE 13. (Top) Cryo-TEM images of (L)-PA-PLX-(L)-PA (PI), (DL/L)-PA-PLX-(DL/L)-PA (PII), and (DL)-PA-PLX-(DL)-PA (PIII) thermogels at 37 °C. (b) Live (green)/dead (red) cell images cultured in the thermogels. The scale bar is 200 μm. Adapted from ref 31 with permission. Copyright 2010 Elsevier.

do not significantly decrease pH during the degradation because the degradation products are neutral amino acids. Therefore, current thermogelling polypeptide block copolymer systems are promising for protein drug delivery. As a model system, an insulin depot was investigated using the PEG-PAF thermogel, and hypoglycemic efficacy was observed for >16 days after a single injection of the insulin formulation into diabetic rats (Figure 12).[28] Several other sustained release systems using this material for protein/peptide drugs are under investigation in our group.

The goal of tissue engineering is to develop a biological replacement for damaged tissues. It requires an integrative understanding of cells, factors, and scaffolds, where the biocompatible scaffold material plays a critical role in developing a successful tissue engineering system.[63] By controlling the secondary structure of a thermogelling polypeptide, the gel could be controlled to have a significant population of nanofibrous structures, similar to a natural extracellular matrix. Chondrocytes cultured in the (DL/L)-PA-PLX-(DL/L)-PA thermogel (three-dimensional (3D) culture) maintained their spherical phenotypes, whereas the chondrocytes cultured in a two-dimensional (2D) plate lost their original spherical phenotype and developed fibroblastic morphologies (Figure 13). Matrigel, a decellularized extracellular matrix of rat tumor tissue, is a commercially available system and was used as a control. (DL/L)-PA-PLX-(DL/L)-PA provided a more compatible microenvironment than Matrigel for in vitro and in vivo culture, and the cells cultured in thermogel of (DL/L)-PA-PLX-(DL/L)-PA expressed biomarkers predictive of articular cartilage, such as sulfated glucoaminoglycan (sGAG) and collagen type II.[31] The modulus of the hydrogel significantly affects the proliferation and differentiation of

cells.[64,65] The modulus could be controlled by varying the initial concentration of (DL/L)-PA-PLX-(DL/L)-PA.[66]

Live cell microscopy is an interesting application for the thermogel because the cells are captured in the transparent gel with an appropriate modulus during the sol-to-gel transition. The thermogel was successfully used as a three-dimensional medium to investigate the cellular pathway of the live cells by microscopy.[67]

Conclusions

This Account summarized the key thermogels developed in our lab, focusing on the control of gel duration, reconstitution time, and degradability. Also, the principles used in the design of each thermogel to solve the problems of each system were described. Thermogelling polypeptide block copolymers were especially emphasized due to their potential for protein drug delivery and tissue engineering applications. The molecular parameters that should be considered in designing a new thermogelling polymer can be summarized as follows. First, the polymer should have a balanced structure of hydrophilicity and hydrophobicity. Not only the ratio of the molecular weight of hydrophobic block to hydrophilic block but also the absolute length of each block is important. For example, thermogelation is observed only when using a low molecular weight form of PEG for a PEG/PLGA system. In addition, the secondary structure of the polypeptide is affected by the molecular weight of each block. Second, the composition of the hydrophobic block is also important. At a fixed molecular weight, the incorporation of hydrophobic components, such as Phe and Leu, facilitates thermogelation of the block copolymer by increasing hydrophobic interactions. The composition of a polypeptide also determines the secondary structure of the polypeptide. When the repeating units have a chiral center, the stereoregular polymer has a greater tendency for thermogelation. Third, topology affects nanoassembly and themrosensitivity, as in the case of PEG-L-PA-DL-PA and PEG-DL-PA-L-PA. Fourth, block copolymers tend to self-assemble in water into spherical micelle or cylindrical micelles, depending on the ratio of hydrophilic block to hydrophobic block length. In addition, the secondary structure of a polypeptide also affects the assembled structure of the amphiphiles. A β-sheeted PEG-L-PA formed nanofibrous structures, whereas α-helical PEG-L-PA formed spherical micelles.[30] Fifth, a dual stimuli-sensitive thermogelling system can be developed for a specific application. A pH-sensitive thermogel can be used for endoscopic therapy if it maintains a sol state in the catheter, which turns into a gel at the target site with a specific pH. Sixth, the degradation mechanism should also be considered. Hydrolysis is the fundamental degradation mechanism of traditional biodegradable polymers such as polyesters, polyanhydrides, polyorthoesters, and polyphosphazenes. In the case of enzyme-degradable polymers, including polypeptides with specific sequences, degradation is accelerated only after the specific in vivo applications. PEG-PAF thermogel was quite stable in phosphate buffered saline; however, it was almost eliminated after 16 days in the subcutaneous layer of rats. Thermogelling polymer can be designed to be degraded by biomolecules. When the biomolecule is related to a specific disease, a disease-responsive drug releasing system can be designed. Seventh, the gel modulus is related to the composition and the block lengths of the hydrophobic block and the hydrophilic block. In the case of 3D stem cell culture, the gel modulus affects the differentiation of stem cells. As the modulus of the gel increases, the stem cells tend to differentiate through processes of neurogenesis, chondrogenesis, and osteogenesis.[65,68] Eighth, gel duration should be correlated with the biofunction of the system. A short-term drug delivery system requires a gel with a short duration. For tissue engineering applications, the tissue growth rate should be matched with the degradation kinetics of the scaffold. As discussed for Pluronics versus PEG-PLGA-PEG systems, the intermolecular interactions and degree of percolation in the gel state affect the gel duration. Control of the topology also affects the gel duration, as in the case of PEG-*g*-PLGA and PLGA-*g*-PEG. In the case of enzymolysis, the availability of specific enzymes and specific sequences affects the degradation of the polymer and thus gel duration. Ninth, as a drug delivery system, the thermogelling system might show an initial burst release of the drug. Specific intermolecular interactions including ionic interactions, hydrophobic interactions, and inclusion complex formation between the drug and polymer can reduce the initial burst. The initial burst during the sol-to-gel transition can be reduced by a preformed gel-injection strategy, where the formulation in a sol state is preheated to form a gel before the injection. In this case, the gel modulus should be low enough to allow the gel injection. Tenth, biocompatibility is a major issue for all types of biomaterials. The pharmaceutical agents or cells should be compatible in the sol state as well as the gel state. Not only the thermogelling polymer, degradation products, residual catalysts, and residual monomers but also the gel modulus and drug–polymer interactions might affect the biocompatibility of the system.

In conclusion, thermogelling polymer aqueous solutions are very promising for drug delivery and tissue engineering applications. In particular, as demonstrated by studies on insulin delivery and 3D culture of chondrocytes, protein drug delivery, tissue engineering, and cell/stem cell therapy are very promising fields for future research using these materials. The molecular parameters described above represent a guideline for designing a new thermogelling system for a specific application.

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FOOTNOTES

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