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In Vitro Evaluation and Dissipative Particle Dynamics Simulation of PLGA-PEG-PLGA

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ABSTRACT: Thermosensitive PLGA-PEG-PLGA triblock copolymers have great potential to be utilized in intravitreal injection. It is important to understand the mechanisms of selfassembled behaviors of triblock copolymers. In this study, dissipative particle dynamics simulation is used to explore the phase behaviors and morphologies of the copolymers. Phase transition, complex viscosities, swelling characteristics, and degradation behavior were investigated. The results suggested that the structures of copolymers were greatly affected by polymer concentration. Phase behavior of copolymers can be modified by alternating polymer size distribution. It exhibited that particle diameter was temperature dependence and polymer concentration can increase micelles numbers in solutions.
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INTRODUCTION

Developing an ideal ophthalmic drug delivery system (DDS) poses unique challenges because of the special anatomy, physiology, and biochemistry of the eye.[1] Current ocular drug delivery systems, of which eye drops represent 90% of all ophthalmic dosage forms, come with substantial disadvantages in terms of uneven distribution of drug concentrations in the anterior (cornea, conjunctiva, sclera, aqueous humor, and iris-ciliary body) when comparing posterior tissues (lens, vitreous, and retina), premature elimination, and other side effects. Consequently, there is a significant effort directed toward developing new drug delivery systems for ophthalmic administration, particularly, in the treatment of serious diseases associated with the posterior parts of the eye.[2,3]

In situ forming hydrogel, as a kind of promising ophthalmic drug delivery system, has been extensively studied during last two decades. These hydrogels are free flowing solutions *in vitro* and respond to small changes in a phenomenon such as temperature, pH, and electrolyte composition *in vivo*, forming physically cross-linked hydrogels by sol-gel phase transition.[1,4–9] The ideal *in situ* hydrogels requires a special phase transition at a desired stage: a solution state for drug dissolved *in vitro* and a gelatin state for drug sustained released *in vivo*. However, many factors such as swelling behavior, mechanical strength of the hydrogel network, biodegradation, and nontoxic nature are important factors at almost every application step.[10,11] It has been reported that many different materials have been used to synthesize the temperature sensitive hydrogels, most of them come with some form of shortcomings. For example, most common synthetic polymers-Pluronics and their derivatives have been shown to be nonbiodegradable, toxic to surrounding tissues and with relatively shorter life *in vivo*. Other biodegradable polymers such as poly (L-lactic acid) (PLLA), poly (D,L-lactic acid) (PDLA), poly(lactic acid-co-glycolic acid) (PLGA), and poly (D,L-lactic acid-co-ε-caprolactone) (PDLA-co-PCL) have also been investigated in recent years. ReGel, poly (lactic acid-co-glycolic acid)-poly (ethylene glycol)-poly (lactic acid-co-glycolic acid) (PLGA-PEG-PLGA), a trademark triblock copolymer, has been reported to be able to carry protein drugs and paclitaxel with sustained release *in vivo*.[12] However, the gelation temperature of ReGel system is lower than room temperature making it to polymerize at room temperature. Thus, it a hard choice for injection administration. An improvement over ReGel has been studied by Qiao's group in which they used higher D,L-lactide/glycolide molar ratio, which resulted in a higher gelation temperature (29°C–36°C) and increased drug solubility.[13]

Although many works have been carried out to investigate the selfassembled behaviors of triblock copolymers, the theoretical

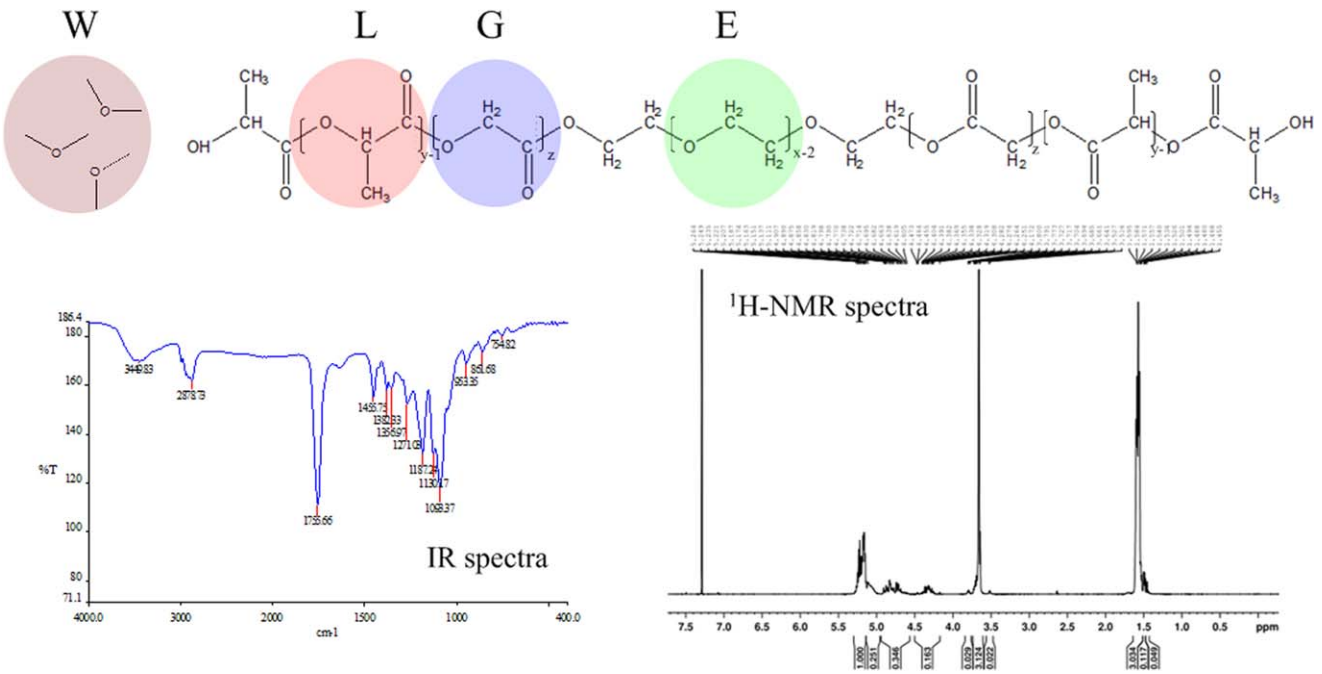

Figure 1. Coarse graining and the ^{1}H-NMR spectra of PLGA-PEGP-PLGA copolymer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

study is relatively scarce. Molecular simulations together with the high performance of modern computers offers a useful way to explore the morphology and other physical properties of the polymers. Recently, dissipative particle dynamics (DPD) simulation has been widely used since proposed by Hoogerbrugge and Koelman about 20 years ago.[14,15] It is a mesoscopic simulation method for complex fluids which can give the correct long-term hydrodynamic behavior of the system.[16–22] It is a well-known fact that copolymer concentration has a great effect on the phase transition as well as swelling and biodegradable behavior, which are intrinsic required properties for vitreoretinal drug delivery. With the aim of better understand the selfassembled behaviors of triblock copolymers, DPD simulation is used to explore the morphologies and polymer distributions.

Meanwhile, the previous studies did not focus on the effect of copolymer concentration on the drug release, polymer swelling, and biodegradable behavior. In order to invesigtae the required properties of hydrogels, such as swelling characteristics and degradation behavior, we synthesized PLGA-PEG-PLGA copolymers by the ring-opening method with D,L-lactide/glycolide molar ratio of 6 : 1. DPD simulation was employed to explain the mechanism of morphological transition. Ganciclovir was taken as the model drug to study the drug release profile. We believe that the results may be beneficial for further explaining the drug release behavior of PLGA-PEG-PLGA copolymer hydrogels *in vitro*.

EXPERIMENTAL

Materials

Polyethylene glycol (PEG 1500) was purchased from Kelong Chemical Reagents Co. Ltd, Chengdu, Sichuan, China. D,L-Lactide and glycolide were purchased from Daigang Bio-Tech. Inc., Jinan, Shandong, China. Stannous octoate was obtained from Sigma (St. Louis, MO, US). Ganciclovir was supplied by Wuyijianyuan Chemical Reagents Co. Ltd., Zhejiang, China. Other reagents were all of analytical reagent grade, purchased from Chuandong Chemical Reagents Co. Ltd, Chongqing, China.

DPD Models and Interaction Parameters

In the DPD simulations, a group of atoms or a volume of fluid is named as bead, which is large on the atomistic scale, but macroscopically small.[23] All beads comply with Newton's equations of motions.[24] The components being used in this work comprised of PLGA-PEG-PLGA copolymers and water. The coarse grained bead-spring models were shown in Figure 1. The molecular structure of PLGA-PEG-PLGA was divided into three types of beads (L, G, and E). Three water molecules are represented as one bead (W). The interaction parameters $\alpha_{ij,}$ shown in Table I, were calculated according to the following equation: $\alpha_{ij} = \alpha_{ii} + 3.27\chi_{ij}$, where χ_{ij} is calculated by Amorphous Cell and Discovery in Materials Studio 4.3 (Accelrys Inc.).[25–27] To avoid the finite size effects, a cubic simulation box of 40 × 40 × 40 Rc^3 with periodic boundary condition is applied in all three directions. The particle density is 3. The integration time step of 0.05 and simulation steps of 40,000 were used for the system to get thermodynamic equilibrium. The main goal of this work is

Table I. The Interaction Parameters Between Beads in DPD Simulations

α_{ij}	*W*	*L*	*G*	*E*
W	78.00			
L	161.30	78.00		
G	149.30	78.04	78.00	
E	114.00	82.96	81.76	78.00

to study the morphologies and microstructures of PLGA-PEG-PLGA at different concentrations from 10% to 30%.

Preparation of PLGA-PEG-PLGA Triblock Copolymers

The PLGA-PEG-PLGA copolymers were synthesized by ring-opening polymerization of D,L-lactide and glycolide with PEG in the presence of stannous octoate.[28] In brief, PEG 1500 (14.58 g) was dried with stirring at 150°C for 2 hr in a three-necked flask under nitrogen atmosphere. D,L-Lactide (5.67 g) and glycolide (1.74 g) were added in the molar ratio of 6 : 1, respectively, to PEG flask and the contents were further stirred for 0.5 hr. Stannous octoate was added after the D,L-lactide and glycolide were dissolved. The crude products were obtained after heating the solutions at 150°C for 2 hr.[13,28] Water-soluble low-molecular weight polymers and unreacted monomers were removed by dissolving the crude mixtures in ice water bath at 5–8°C followed by heating up to 80°C and then allowed to precipitate. For further purifications, precipitated polymers were redissolving in ice water and purified by repeating the above steps until the supernatant of the solution showed neutral pH value. Finally, the purified products were lyophilized and stored at −20°C.

Measurement of Phase Diagram

Sol-gel transition behavior was investigated by inverted test tube method.[29–31] Two milliliter samples (5%, 10%, 15%, 20%, 25%, 30%, w/v) were prepared by dissolving the polymer in distilled water in a 4 mL vial and equilibrated at 8°C for 12 hr. The solutions were warmed in a water bath, increasing temperature from 10°C to 60°C in 1°C interval steps. The vials containing samples at a given temperature were equilibrated for at least 20 min. The sample was regarded as gel phase in the case of no flow within 30 s of inverting the vial.

Measurement of Particle Size Distributions

Samples (1%, 5%, 10%, 15%, 20%, 25%, 30%, w/v) were prepared and equilibrated at 8°C for 12 hr. Dynamic light scattering (DLS) methods were used to measure particle size distributions ranging from 15°C to 45°C by Zetasizer Nano ZS 90 instrument (Malvern, UK).[32]

Measurement of Rheological Behaviors

Polymer solutions (20%, 25%, 30%, w/v) were prepared and equilibrated at 8°C for 12 hr. Samples (1.5 mL) were taken and rheological behaviors were investigated by Bohlin rotational rheology instrument (Malverm, UK) at 1 Hz, with increasing temperatures by 1°C per min from 5°C to 60°C.[3,33] The polymer solution was placed between parallel plates of 40 mm diameter and a gap of 0.5 mm. The sample plates were covered carefully to minimize solvent evaporation. The experiments were repeated three times at each condition and the results presented are averages.

Measurement of Swelling Index

Samples (20%, 25%, 30%, w/v) were prepared as mentioned above and equilibrated at 8°C for 12 hr. Three blanks for each sample were prepared as well. Samples in bottles were equilibrated in water bath at 37°C for 15 min. When the gel was formed, the gels were weighed (Wi) and PBS (3 mL, 37°C) was dropped into the gel systems slowly. The bottles were sealed to avoid solvent evaporation. The samples were equilibrated at 37°C for a given time (50 rpm/min), the gel was reweighed after removing the upper solution supernatant (Wt). Swelling index was calculated by the following equation: SI = Wt/Wi .[11,34]

Measurement of Biodegradation

The degradability rate of PLGA-PEG-PLGA copolymers was determined by weight measurement *in vitro*.[35] Samples (20%, 25%, 30%, w/v) were prepared and equilibrated at 8°C for 12 hr. Three blanks for each sample were prepared as well. The initial copolymers were weighed (Wi). Samples in bottles were equilibrated in water bath at 37°C for 15 min. When the gel was formed, PBS (3 mL, 37°C) was dropped to the gel slowly. The bottles were sealed to avoid solvent evaporation. After shaking equilibration at 37°C (50 rpm/min), the solutions of samples were removed at a given time. The gels of copolymers were lyophilized until constant weight. The percentage of remaining gels can be calculated by following equation: [Wi −(Wd − Wo − Wp)]/Wi × 100%. (Wi: initial weight of gel; Wd: weight of the dry bottle; Wo: initial weight of the bottle; Wp: weight of PBS.)

Measurement of Drug Release Behaviors

Drug release behaviors were measured by the modified methods reported previously.[30] Ganciclovir (5 mg) was added to 1 mL of copolymer solutions (20%, 25%, 30%, w/v) and equilibrated at 37°C for 15 min. Eight milliliter PBS (37°C) was dropped slowly to the surface of gels after the sol-gel transition. The system was sealed to avoid evaporation of reagents and incubated in a shaking bath (50 rpm/min) at 37°C. At designated time intervals, a 2 mL aliquot was taken from the release media. The same amount of fresh buffer was then added in order to maintain the sink condition. 1 mL of collected sample was then diluted to 10 mL in PBS. The absorbance of samples was determined at 252 nm. Accumulated release percentage of ganciclovir was determined as:

$$Q\,(\%) = \frac{C_n \cdot V + V_i \sum_{i=0}^{n-i} C_i}{m_{\text{drug}}} \times 100\%, \qquad (1)$$

here, Q (%) was the amount of accumulated release drugs. V (mL) was the total volume of samples. Cn (mg/mL) and Vi (mL) were the concentration and volume of samples taken at n and i time point. m_{drug} (mg) was the mass of drug in gels. The number of times of drug release media replacements was numbered as n. The release data were analyzed by the classic models (Zero-equation, First-equation and Higuchi equation).

RESULTS AND DISCUSSION

Characterization of PLGA-PEG-PLGA Triblock Copolymers

The typical ^{1}H-NMR spectrum of the copolymer synthesized (Figure 1) was similar to the reported spectrum and all the signals were assigned on the spectrum.[36,37] The characteristic signals appearing at 5.187, 4.338, 1.571, 4.738, and 3.661 ppm are assigned to CH of D,L-LA (peak area = 1.000), CH of D,L-LA (peak area = 0.163), CH_3 of D,L-LA (peak area = 3.034), CH_2 of GA (peak area = 0.346), and CH_2 of PEG (peak area = 3.124). Due to the random copolymerization of glycolide and lactide, there were complicated splits in these peaks.[37] Average

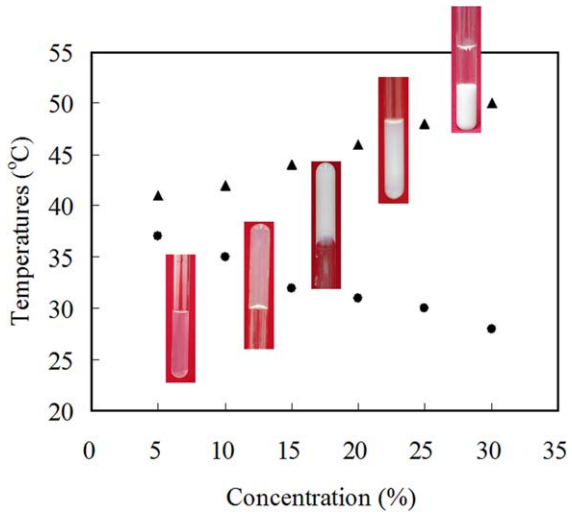

Figure 2. Phase diagrams of PLGA-PEG-PLGA copolymers ranging from 5% to 30% (w/v). Triangle: Sol-gel temperature. Circle: Gel-precipitate temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

molecular weight of copolymers was 8337, calculated by end-group analysis using ^{1}H-NMR spectrum.[29,32] The peak areas of CH of D, L-LA, CH_2 of GA, CH_2 of PEG, and CH_3 of D, L-LA were substitutes into the following equations and the average molecular weight obtained was 8337.

$$y-1 = A_{\text{CH3 of D,L-LA}}/A_{\text{CH of D,L-LA}} \quad (2)$$

$$2z/(y-1) = A_{\text{CH2 of GA}}/A_{\text{CH of D,L-LA}} \quad (3)$$

$$4x/(y-1) = A_{\text{CH2 of PEG}}/A_{\text{CH of D,L-LA}} \quad (4)$$

$$\text{Mn} = y \times 2 \times 72 + z \times 2 \times 58 + x \times 1500 = 8337.$$

The FTIR spectra of the PLGA-PEG-PLGA were shown in Figure 1. The peak at 3449.83 cm^{-1} is associated with the OH at the end of the copolymers, the peaks at 2878.73 cm^{-1} is due to stretching vibration band of CH. The peaks at 1755.66 and 1455.75 cm^{-1} is associated with stretching vibration band of C=O and C–C, respectively. The static water contact angle gives the information about the hydrophobic/hydrophilic of polymers. The contact angle of PLGA-PEG-PLGA copolymer solution was 33.8°, indicating the PLGA-PEG-PLGA copolymers can better the surface energy of retinal and mimic the surface properties of native tissues.[13]

Phase Diagram of PLGA-PEG-PLGA Copolymers

PLGA-PEG-PLGA triblock copolymers present three physical states during the transitions: solution, gel, and precipitate from 10°C to 60°C.[31] In this study, two temperatures are defined: low transition temperature from sol to gel and upper transition temperature from gel to precipitate. Figure 2 showed the phase diagrams with different polymer concentrations, a lower transition temperature curve from sol to gel and an upper transition temperature curve from gel to precipitate. The low transition temperatures of polymers (from 5% to 30%, w/v) decreased from 37°C to 28°C. While, the upper transition temperature increased from 41°C to 50°C at the same concentration, suggesting that higher polymer concentrations lead to lower sol-gel transition temperatures and higher upper transition temperatures, which enlarged the gel zones. As for samples with the same molecular weight and composition, transition temperatures exhibited concentration-dependence. The equations of transition temperatures also were fitted in our study. It suggested a linear relationship between transition temperature and polymer concentration.

$$Y(\text{low transition temperature}) = -0.3486z + 38.267 \quad (R^2 = 0.9694)$$

$$Y(\text{upper transition temperature}) = 0.3714z + 38.667 \quad (R^2 = 0.9922)$$

The PLGA-PEG-PLGA triblock copolymers synthesized in our study with concentrations from 5% to 30% had transition temperatures from 28°C to 37°C. The gel window covered the physiological temperature (37°C). The transition temperatures located in the scopes between the room temperature and body temperature, indicating the drugs could be mixed with the copolymer solutions at lower temperature, protecting drug away from denaturing, aggregation, and any undesired chemical reaction, and incorporated in the hydrogel after injection administration for ophthalmic drug delivery.[8,30]

DPD Simulation of PLGA-PEG-PLGA Copolymers

It is well known that at the right balance of hydrophilic and hydrophobic moieties, triblock copolymers can spontaneously self-assemble into micelles. In the DPD simulation, phase separation and polymer micelles were shown in Figure 3 for the copolymer concentration of 5%, 10%, 15%, 20%, 25%, and 30%, respectively. The water molecules were not shown in order

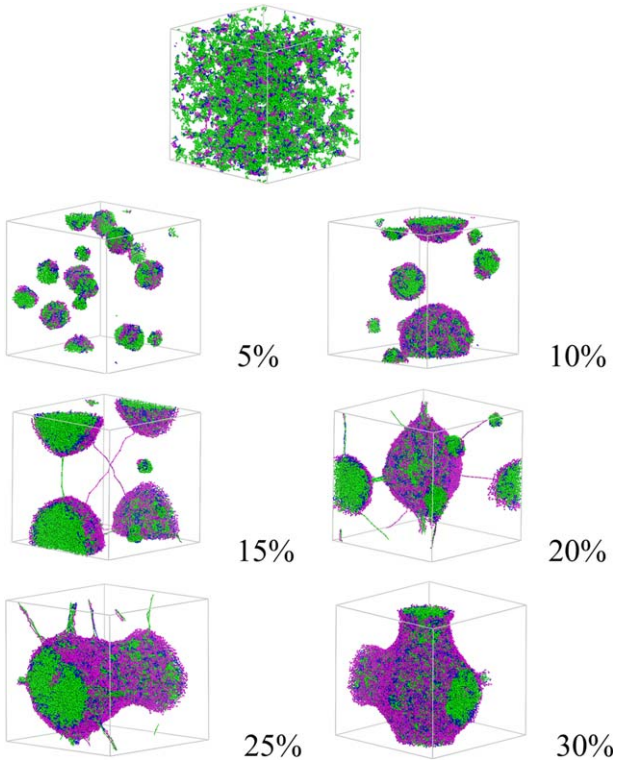

Figure 3. Morphologies of PLGA-PEG-PLGA copolymers at different concentrations (Blue beads: G; Green beads: L; Purple beads: E). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

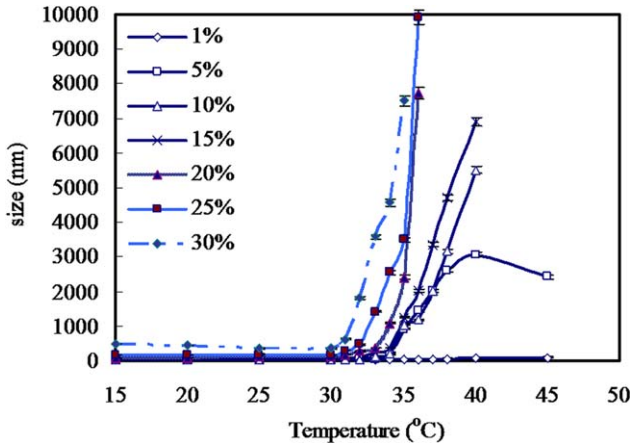

Figure 4. Particles size distribution of PLGA-PEG-PLGA copolymers ranging from 1% to 30% (w/v). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

to display the molecular arrangement of the PLGA-PEG-PLGA. The beads of *W*, *L*, *G*, and *E* were in homogeneous phase at the beginning. With the simulation time increasing, the microphase separations were observed and formed different structures at different compositions finally. The hydrophobic D,L-LA, GA of PLGA segments (L and G beads) distributed homogeneously in the core of micelles based on the hydrophobic interactions. The hydrophilic PEG segments (E beads) distributed on the surface. Several small spherical micelles were observed when the copolymer concentration was low (5% and 10%) in the simulation. With the polymer concentration growing up to 20%, the small spherical particles united to be a larger one. When the polymer concentration was up to 25% and 30%, the aggregating morphology was not spherical and a columnar structure was formed, which ensured that the area of hydrophobic groups contacting with water was minimal and the system were kept stable.[38,39] Thermoreversible gel-precipitate transition is closely related with intrinsic changes in micelle properties.[40] The DPD simulation results showed qualitatively the changes of the aggregating morphology at different concentration. PLGA-PEG-PLGA triblock copolymer micelles are formed under strong hydrophobic attraction and with bridging connections between micelles because of the diffusion of hydrophobic PLGA blocks into different micelles in aqueous environment.[29] The core-shell structure of micelle is consisted of a hydrophilic PEG outer shell and hydrophobic PLGA inner core. Thus, the free energy of hydration is decreased. Due to higher micelle concentrations resulted from higher polymer concentration, the sol-gel transition is induced by the packing of aggregated micelles at lower temperature.[31]

Particle Size Distributions

As potential injectable drug systems, phase behavior of PLGA-PEG-PLGA copolymers can be modified by alternating the polymer size distribution. In this study, particle diameter was found to be temperature dependence and polymer concentration can increase micelles numbers in solutions. It has been reported that copolymer micelles, selfassembled in polymer solutions, were composed of hydrophobic PLGA cores and hydrophilic PEG shells below the sol-gel transition temperature, but may form bridging micelles with increasing concentration and temperature.[41] It was indicated from the DPD simulations in this study. Figure 4 described the micelle size and size distribution as a function of temperature. As shown in Figure 4, at the same temperature, the higher concentration of copolymers, the larger scope of particle size distribution. For example, particle size of 25% copolymers was 5.85 larger than 1% at 15°C. And the number increased up to 16.34 when comparing 30% with 1% solutions at the same temperature. The diameters of copolymers micelles in 30% can reach to 494.00 nm at 15°C. It indicated that micelles with increasing aggregation numbers packed each other, resulting in the increase of copolymer diameters at high polymer concentration.[32] Therefore, the interactions among micelles were enhanced, resulting in the sol-gel transition in low temperatures. When came to the same concentration, particle sizes of copolymer micelles enlarged with the increasing temperatures. An abrupt size increasing may occur at some certain temperature (except 1%, w/v). Micelles assembly with PLGA-cores and PEG-shells were formed. When temperatures increased from 30°C to 40°C, the diameters of aggregated micelles enlarged, resulting from the interactions between hydrophobic PLGA blocks and other micelles.[29] Since hydrogen bonds between hydrophilic PEG molecules and water molecules were in dominate in the aqueous solution, resulting in their dissolution in water, copolymers present sol state at low temperatures. Meanwhile, the terminus of hydrophobic PLGA segments may interact with other micelles to form micelles. As the temperature increases, the hydrogen bonding between PEG blocks becomes weaker, while hydrophobic forces among the hydrophobic PLGA segments strengthened leading to sol-gel transition.[12] When the temperature rose up to 40°C, polymers size decreased, since the micelles shrunk strongly and water in micelles was pumped out.

Measurement of Rheological Behaviors

The viscosity of a polymer solution can evaluate its resistance to flow, which is a complex function of its molecular weight, its concentration, as well as of the temperature and the shear stress.[1] As be injection administration for ophthalmic drug delivery, complex viscosity of PLGA-PEG-PLGA copolymers (20%, 25%, and 30%) was measured [Figure 5(A)]. The results showed that copolymer solutions exhibit a sol-like behavior at temperature below 25°C (room temperature) and at this condition, the complex viscosity (η^*) was independent of polymer concentration. However, when the temperature increased above 30°C, η^* increased sharply and the complex viscosities were various for different copolymer solutions. The corresponding temperatures for abrupt complex viscosities increment were different for each polymer concentration here studied. η^* increased at a faster rate with increasing temperature. In addition, higher polymer concentration induced lower temperature phase transition and stronger gel rigidity. At 37°C, the complex viscosities of 20%, 25%, and 30% copolymer samples were 56.7, 106.00, and 132.47 Pa.s. It achieved the maximum values of 114.58, 155.52, and 195.68 Pa.s for these samples at corresponding temperatures, respectively. Polymer solution with 30% concentration had the highest complex viscosity.

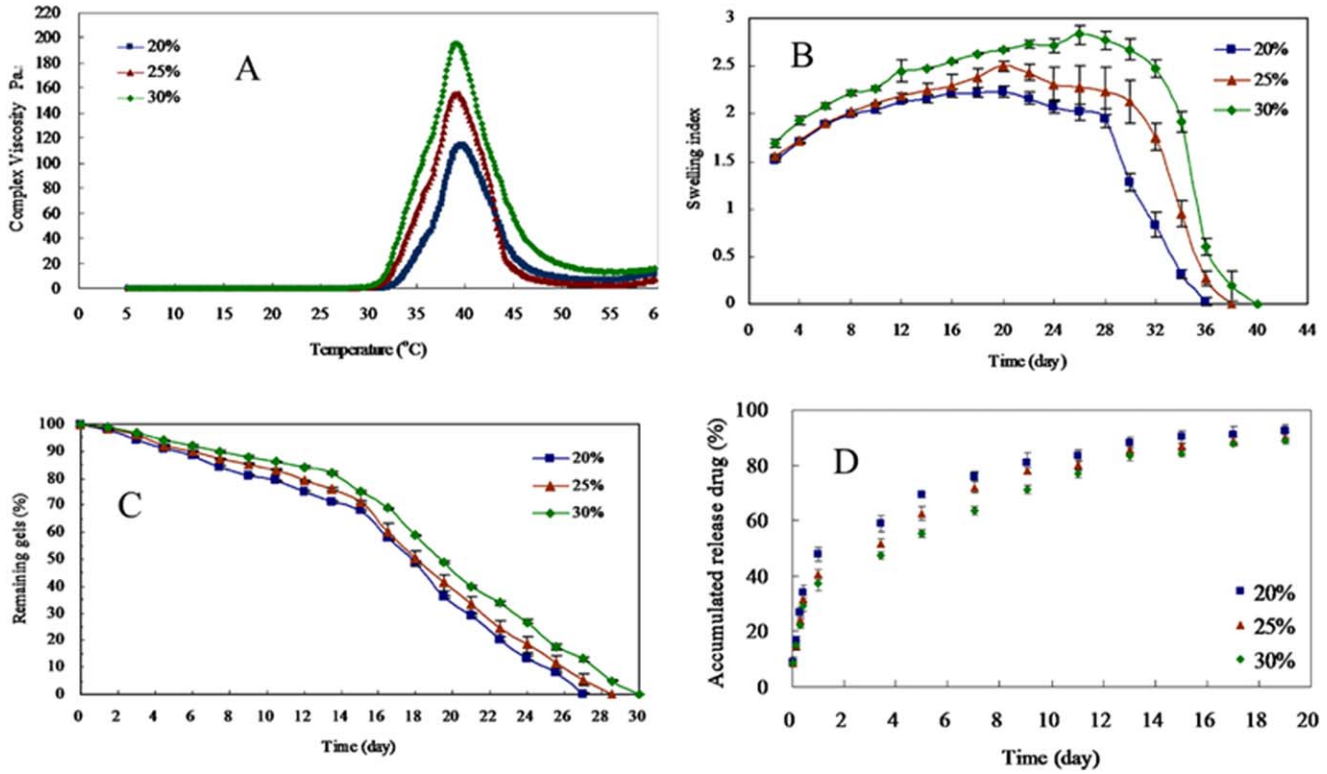

Figure 5. (A) Complex viscosities of PLGA-PEG-PLGA; (B) Swelling index of PLGA-PEG-PLGA; (C) Degradation profiles of PLGA-PEG-PLGA; (D) Ganciclovir release from PLGA-PEG-PLGA hydrogels, free drug as a control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The complex viscosity describes the relationship between the dynamic viscosity and the out-of-phase viscosity, or imaginary part of the complex viscosity. Only some bridging micelles of PLGA-PEG-PLGA copolymers are formed, which are not stable due to the low hydrophobicity of PLGA below the sol-gel transition temperature.[42] With temperatures increase over sol-gel transition temperature, a bridged micelle network is formed resulting from the increasement of hydrophobicity of the PLGA segment.[33,42] The elasticity of copolymers takes predominant, leading to gelatin. Generally, solutions with complex viscosity <1 Pa.s are required to go through needles smoothly at room temperature.[43] In this study, the complex viscosities of 0.03, 0.06, and 0.18 Pa.s for PLGA-PEG-PLGA copolymer solutions with 20%, 25%, and 30% were obtained, respectively, at 25°C, suggesting all samples can be administrated by injection at room temperature.

Measurement of Swelling Index

Swelling index of PLGA-PEG-PLGA hydrogels was measured by weight analytical method at 37°C [Figure 5(B)]. PLGA-PEG-PLGA hydrogels of 20% and 25% had been increasing swelling indexes in the first 16 days that decreased after the 17th day. A sharp decreased in swelling index occurred on and after the 21st day. The swelling index of 30% sample, however, increased before the 20th day and decreased abruptly on the 26th day, indicating that water may have permeated into the hydrogel network at an initial stage and hence then it swelled, however degradation rarely occurred. The swelling index increased until a balance between swelling and degradation was achieved. Then, degradation became dominant and swelling index of hydrogels decreased down to zero as the time increased, resulting in the collapse of hydrogel networks. Copolymer concentration is another important parameter for hydrogel's swelling behavior with higher concentrations resulting in more absorbed water that in turn may adversely affect the balance between swelling and degradation. We found that hydrogels swelling behaviors took dominated in 20 days at 37°C, beneficial for sustained drug delivery in intravitreal application.

Measurement of Biodegradation

Degradation is one of the most important features of intravitreal injection hydrogels. Figure 5(C) showed the PLGA-PEG-PLGA hydrogels degradation process. The slope of curves after 16 days was higher than before, indicating fast degradation rate of copolymers. The rate of degradation of the copolymers was affected by their concentration in the samples. The degradation periods of PLGA-PEG-PLGA hydrogels were 27 days, 28 days, 30 days for solutions at 20%, 25%, and 30%, respectively. The reason for this can be attributed to higher concentration of the copolymers that can result in more closely connected micelles formation, thereby strengthening the interaction among the polymer. Due to the smaller size of pores in hydrogels, water cannot permeate into the hydrogels freely, resulting in the lower degradation speed of hydrogels.[36,43]

Due to the balanced capacity of copolymer hydrogels, other studies have suggested the drug system degradation did not have a direct relationship with the polymer concentration.[36] This study, however, found that biodegradation rate of the hydrogels was affected by the copolymer concentration. Owing to the core-shell structure of PLGA-PEG-PLGA micelles and micelle-bridges, water permeability was various for different hydrogel systems. At the higher concentration of copolymer solutions, generally, more and closely packed micelles were formed, resulting in the difficulty for water to permeate into the hydrogel. Thus, the biodegradation rate can be slowed down considerably. Polymer degradation is also affected by other parameters, such as the hydrogels' size, shape, structure, composition as well as the rate of water content, and nature of enveloped drugs.[36]

Measurement of Drug Release Behaviors

Ideal vitreoretinal drug delivery systems should release drugs steadily and slowly. Drug release profiles are influenced by many factors, such as diameters of hydrogel pores, biodegradation of systems, hydrophobicity, drug concentration, and the interaction between hydrogels and enveloped drugs.[44] In this study, we chose ganciclovir (GVC) as the model drug to study the release behavior *in vitro*. The release profiles of ganciclovir from PLGA-PEG-PLGA hydrogels are shown in Figure 5(D). About 47% of ganciclovir were released from the system in the first 24 hr, over 50% drug released successively in the next 15 days, followed by a flat curve. It was believed that the huge initial drug release was due to GCV loosely bounded on the surface or embedded in the surface of the hydrogels.[45] However, the expulsion of the aqueous phase resulting from contraction of micelle system volume during sol-gel transition was also responsible for the initial burst release of thermosensitive *in situ* forming hydrogel.[13] It was found that high concentrations of polymers (e.g., 30% copolymers) can suppress the initial drug burst when comparing with low concentrations (e.g., 20% copolymers). Release rate constants and correlation coefficients were calculated for zero-order, first-order, and Higuchi's equations. The best fit of release kinetics with higher correlation coefficients was achieved with first-order release kinetics ($r = 0.9877$). Drug release from the hydrogels was dominated by diffusion due to low matrix erosion rate initially. At a later stage, it was a combination of diffusion and degradation in such a biodegradable hydrogel.

It is a challenge to model drug release from *in situ* forming hydrogels. Some parameters should be carefully taken into account. The distribution of drugs in copolymer micelles is one of the key parameters affecting drug releasing profiles.[31,43] The hydrophobic drug tends to partition into the hydrophobic PLGA domain of the hydrogel and only smaller amount partitions into the hydrophilic PEG domain, while the hydrophilic drug tends to partition into the hydrophilic PEG domain.[12] Ganciclovirs was enveloped in both hydrophilic cores and hydrophobic shells of micelles. When water permeated into hydrogels, drugs on the surface diffused into surrounding medium, resulted in the initial burst drug release. Meanwhile, sol-gel transition may result in shrinkage of hydrogel and thus, water was removed from the system. In this case, initial burst release of ganciclovir is induced as well. As shown in Figure 5(D), when comparing to the 20% and 25% hydrogels, 30% hydrogels have lower ganciclovir releasing rate. It is a well-known fact that gels have a cross-connected network of pores.[46,47] It is easy to form micelles that pack closely with each other in the case of high concentration of polymers. Thus, size diameters of pores in gels could be narrowed, resulting in the slow diffusion out of drug loaded in inner core.[36–44] Meanwhile, since water cannot permeate into the gels easily, hydrogel biodegradation can also be slowed down, therefore, decreasing overall drug release rate.

CONCLUSIONS

Thermosensitive PLGA-PEG-PLGA copolymers as an ideal candidate for intravitreal drug system were investigated in this paper. The properties of copolymers were measured. DPD simulation was employed in an attempt to better understand the morphologies and microstructures of PLGA-PEG-PLGA copolymers with different concentration. Since the phase transition temperatures of copolymers ranged from 28°C to 37°C, PLGA-PEG-PLGA copolymers entrapped drugs at room temperature as free flow solutions and transformed to gelatins at body temperatures (37°C) after injection. The results of DPD simulations indicated that micelles bridges were formed at higher concentration, lead to low phase transition temperatures of copolymers. The complex viscosities of 0.03, 0.06, and 0.18 Pa.s for PLGA-PEG-PLGA copolymer solutions with 20%, 25%, and 30% were obtained, respectively, at 25°C, suggesting it can be administrated by injection at room temperature. The swelling index of hydrogels was steady within 20 days. Biodegradation and erosion of hydrogels took dominant effect afterward. The degradation phases of PLGA-PEG-PLGA copolymers ranged from 27 to 30 days. Polymer concentration as well as the interaction between model drugs and hydrogels significantly affected the degradation time. Ganciclovir was taken as model drugs to evaluate the drug release behaviors from the hydrogels. In this study, ganciclovir was released from the PLGA-PEG-PLGA hydrogels in 18 days successively *in vitro*. This paper took PLGA-PEG-PLGA as an example to investigate the required properties of ideal intravitreal injection copolymers and may offer a standard role to test thermosensitive polymers.

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