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Injectable biodegradable temperature-responsive PLGA–PEG–PLGA copolymers: Synthesis and effect of copolymer composition on the drug release from the copolymer-based hydrogels

Mingxi Qiao[a], Dawei Chen[a,*], Xichen Ma[b], Yanjun Liu[b]

[a] *School of Pharmacy 42 mail box, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, Liaoning Province, PR China*
[b] *Dalian Institute of Light Industry, 116034, No. 1, Qinggongyuan, Ganjingzi District, Dalian, Liaoning Province, PR China*

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Abstract

Injectable biodegradable temperature-responsive poly(DL-lactide-co-glycolide-b–ethylene glycol-b-DL-lactide-co-glycolide) (PLGA–PEG–PLGA) triblock copolymers with DL-lactide/glycolide molar ratio ranging from 6/1 to 15/l were synthesized from monomers of DL-lactide, glycolide and polyethylene glycol and characterized by ^{1}H NMR. The resulting copolymers are soluble in water to form free flowing fluid at room temperature but become hydrogels at body temperature. The hydrophobicity of the copolymer increased with the increasing of DL-lactide/glycolide molar ratio. In vitro dissolution studies with two different hydrophobic drugs (5-fluorouracil and indomethacin) were performed to study the effect of DL-lactide/glycolide molar ratio on drug release and to elucidate drug release mechanism. The release mechanism for hydrophilic 5-fluorouracil was diffusion-controlled, while hydrophobic indomethacin showed an biphasic profile comprising of an initial diffusion-controlled stage followed by the hydrogel erosion-dominated stage. The effect of DL-lactide/glycolide molar ratio on drug release seemed to be dependent on the drug release mechanism. It has less effect on the drug release during the diffusion-controlled stage, but significantly affected drug release during the hydrogel erosion-controlled stage. Compared with ReGel system, the synthesized copolymers showed a higher gelation temperature and longer period of drug release. The copolymers can solubilize the hydrophobic indomethacin and the solubility (13.7 mg/ml) was increased 3425-fold compared to that in water (4 μg/ml, 25 °C). Two methods of physical mixing method and solvent evaporation method were used for drug solubilization and the latter method showed higher solubilization efficiency.

Keywords: Temperature-responsive; Injection; Drug release; Hydrogel

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[*] Corresponding author. Tel.: +86 24 23843711 3687; fax: +86 24 83890024.
E-mail address: qiaomingxi@163.com (M. Qiao).

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1. Introduction

Temperature-responsive polymers have become increasingly attractive as carriers for the injectable drug delivery systems over the past decade. These polymers respond to small changes in temperature stimuli and form physically crosslinked hydrogels by sol–gel phase transition. A class of triblock copolymers (Pluronics or Poloxamers) composed of poly(ethylene glycol-b-propylene glycol-b-ethylene glycol) exhibit reversible sol–gel transition behavior in aqueous solution (Brown et al., 1991). Poloxamer 407 hydrogels have been used for the sustained delivery of several drugs including tridecapeptide melanotan-I (MT-I) (Bhardwaj and Blanchard, 1996), mitomycin C (Miyazaki et al., 1992), interleukin-2 (Johnston et al., 1992) and urease (Fults and Johnston, 1990). Poloxamer 407 hydrogels showed a sustained release of human growth hormone (rhGH) in vitro for 60–72 h and for a week in vivo following intramuscular and subcutaneous injections (Manohar et al., 1997). Use of Poloxamer hydrogel would not only provide a sustained release formulation but also enhance the stability of drugs (Wang and Johnston, 1995; Katakam et al., 1995). The release rate of drug from Poloxamer hydrogel can be modified by mixing additives such as methylcellulose, hydroxypropyl methylcellulose and polycaprolactone. However, Poloxamers are not biodegradable and the formed hydrogels are dissolved at the injection site in a few days at most. Furthermore, application of the high concentration (>16%, w/w) of Poloxamers may show toxicity particularly intended for intraperitoneal administration (Wout et al., 1992; Johnston and Palmer, 1993).

New injectable biodegradable polymers possessing reverse gelation properties have been reported (Cha et al., 1997; Jeong et al., 1997; Rathi et al., 2000; Rathi and Zentner, 1999). These polymers are triblock copolymers composed of A-blocks and B-blocks arranged as ABA or BAB type, where A is polyethylene glycol (PEG) and B is poly(DL-lactide-co-glycolide, PLGA). They are soluble in water at or below room temperature but become hydrogels at the injection site, forming depots that slowly degrade over a period of 4–6 weeks (Jeong et al., 1999). As the polymers are biodegradable, they obviate the need for removal of the carrier after the drug depot exhausted. Release of several drugs from the PLGA–PEG–PLGA triblock copolymers hydrogel (ReGel®) including protein drugs and paclitaxel was reported (Zentner et al., 2001; Kim et al., 2001). ReGel is the trademark of the PLGA–PEG–PLGA copolymers, which the PEG has molecular weight of 1000 and DL-lactide/glycolide molar ratio is 3. The copolymers have the ability to solubilize poorly soluble paclitaxel and stabilize liable protein drugs and provide a sustained release of drugs ranging from 1 to 6 weeks. However, the effect of copolymer composition on the drug release from the copolymer-based hydrogels and hydrogel erosion has not been reported until now.

In this paper, temperature-responsive PLGA–PEG–PLGA triblock copolymers which have the different DL-lactide/glycolide molar ratio from ReGel (ranging from 6/1 to 15/1) were first synthesized and found to possess the thermoreversible gelation character. The influence of DL-lactide/glycolide molar ratio on the drug release from the copolymer-based hydrogels and hydrogel erosion were investigated. The release data of drugs were also compared with results obtained from the ReGel system. In addition, the method of solubilization of hydrophobic drugs within the copolymer micelles was explored.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG 1500) was purchased from Shanghai Pudong Gaonan Chemical Corporation. DL-Lactide and glycolide were purchased from China Rehabilitation Research Center CONAN Polymer R&D Center and used without further purification. Stannous 2-ethylhexanoate was obtained from Sigma (US). 5-Fluorouracil was supplied by Anhui Sinoi implant Hitech & Sicence Co. Ltd. Indomethacin was obtained from North China Pharmaceutical Company Ltd.

2.2. Synthesis of PLGA–PEG–PLGA triblock copolymers

Under nitrogen atmosphere, polyethylene glycol 1500 was dried in a three-necked flask under vacuum and stirring at 120 °C for 2 h. A total of 50 g of DL-lactide, glycolide plus PEG were used for the polymerization. Copolymerization in the bulk state was carried out with various molar ratios of DL-lactide/glycolide (6/1, 10/1, 15/1) and the weight ratio of PEG was adjusted to 30% (w/w). Stannous 2-ethylhexanoate (0.2%, w/w) was added into a vigorously dried polymerization tube followed by the addition of DL-lactide, glycolide and PEG. Then the tube was sealed under vacuum. The sealed tube was immersed and kept in an oil bath thermostated at 150 °C for 8 h. The tube was subsequently broken and the product was dissolved in water. After completely dissolved, the copolymer solution was heated to 80 °C to precipitate the copolymer and to remove the water-soluble low-molecular-weight copolymer and unreacted monomers. The supernatant was decanted to obtain the precipitated copolymer. The process was repeated three times to purify the copolymer. The resulting copolymer was dried under vacuum at room temperature until constant weight. The copolymer nomenclature was designated PLGA–PEG–PLGA(X/Y), where X/Y is the molar ratio of DL-lactide and glycolide.

2.3. Gel permeation chromatography (GPC)

The molecule weights of the PLGA–PEG–PLGA copolymers were determined using a Shimadzu LC-10AD HPLC pump and Shimadzu RID-6A refractive index detector (Kyoto, Japan) coupled to a Hewlett Packard Plgel column. Tetrahydrofuran was served as solvent with a flow rate of 1 ml/min. The molecular weights of the copolymers were determined relative to polystyrene standards.

2.4. ^{1}H nuclear magnetic resonance (NMR)

^{1}H NMR spectra of PLGA–PEG–PLGA copolymers were obtained in $CDCl_3$ using a NMR instrument (Bruker ARX-300) at 300 MHz. The DL-lactide to glycolide ratio of the copolymer was determined by ^{1}H NMR.

2.5. Measurement of gelation temperature (Yong et al., 2001)

A 20-ml transparent vial containing 2.6 g of magnetic bar (cylinder, 10 × 5 mm i.d.) and 10-g water solution of PLGA–PEG–PLGA copolymer was placed in a water bath. The solution was heated at a constant rate of 2 °C per minute with constant stirring (200 rpm). When the magnetic bar stopped stirring due to gelation of the solution, the temperature read from the thermometer was determined as the gelation temperature.

2.6. Indomethacin solubilization by PLGA–PEG–PLGA copolymers

2.6.1. Solvent evaporation method (Lavasanifar et al., 2001)

Excess amounts of indomethacin and PLGA–PEG–PLGA copolymers were both dissolved in acetone in a round-bottomed flask. Acetone was removed under vacuum at 50 °C. After the acetone was completely removed, the copolymer/drug film was hydrated to obtain the copolymer solution (25%, w/w). The suspensions were centrifuged for 10 min and the indomethacin concentration in supernatant was analyzed using HPLC. For the chromatographic conditions, see Section 2.8.

2.6.2. Physical mixing method

Excess amounts of indomethacin was mixed with the PLGA–PEG–PLGA copolymer solutions by vortexing for 30 s and placed in the shaking water bath at 25 °C with shaking for different time periods to establish an equilibration time. The suspensions were centrifuged for 10 min and the concentrations of indomethacin in the supernatant were analyzed by HPLC.

2.7. Preparation of drug-loaded PLGA–PEG–PLGA copolymer solutions

The PLGA–PEG–PLGA copolymers were dissolved in the distilled water containing 5-fluorouracil to make 25% (w/w) copolymer solution with 0.5% (w/w) drug loading. Indomethacin was incorporated into PLGA–PEG–PLGA copolymers with the same drug loading as 5-fluorouracil using solvent evaporation method.

2.8. In vitro erosion studies

The copolymer solutions (25%, w/w, 1 ml) were placed into test tubes and incubated at 37 °C. After 2 min, 3 ml of phosphate buffer (pH 7.4) was added to the test tube and the surface area exposed to the release medium was 0.50 cm^2. The tubes were shaken at 50 rpm. At sampling times, the release medium of each test tube was transferred to the pre-weighed vial and evaporated until constant weight. The amount of copolymer erosion was calculated as: $W_\mathrm{d} - W_\mathrm{o} - W_\mathrm{p}$, where W_d is the weight of vial after drying, W_o the original weight of the vial and W_p the weight of phosphate salt.

2.9. *In vitro release studies*

The copolymer solutions containing the model drug (1 ml) were manipulated according to Section 2.8. At sampling times, the release medium was replaced with the same amount of fresh buffer in order to maintain sink conditions and subjected to high-performance liquid chromatography (HPLC) for analysis. HPLC (SPD-10Avp, Shimadzu) was equipped with a C_{18} column (5 μm particle, 150 × 4.6 mm i.d., Diamonsil$^{\mathrm{TM}}$). The mobile phase was methanol–water with a ratio of 5:95 (v/v) for 5-fluorouracil and methanol–sodium acetate anhydrous buffer (20 mM, pH 4.6) 80:20 (v/v) for indomethacin. UV–Vis detection at 265 and 320 nm was used for the analysis of 5-fluorouracil and indomethacin, respectively. The flow rate of mobile phase was 1 ml/min.

The release data were evaluated by model-dependent methods. Drugs diffusion from the polymer matrix can be described by Eq. (1). The equation can be simplified as Eq. (2) or (3) depending on the value of M_t/M_∞:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left[\frac{-D(2n+1)^2\pi^2}{l^2} t\right] \tag{1}$$

$$\frac{M_t}{M_\infty} = 4\sqrt{\frac{Dt}{\pi l^2}}, \qquad \frac{M_t}{M_\infty} \le 0.6 \tag{2}$$

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\exp\left[\frac{-\pi Dt}{l^2}\right], \qquad \frac{M_t}{M_\infty} > 0.4 \tag{3}$$

where M_t is defined as the mass of the drug released at time t, M_∞ is the mass of drug released as time approaches infinity, and D and l are the diffusion coefficient of the drug and the thickness of the device, respectively.

2.10. *Statistics*

Statistical analysis of the effects of increasing DL-lactide/glycolide ratio (6/1, 10/1, 15/1) on the gelation temperature, rate of erosion and drug release was performed using a one-way ANOVA at all different pints. In all cases, post hoc comparisons of the means of individual groups were performed using Tukey's honestly significant difference test. A significance level of $P < 0.05$ denoted significance in all cases.

3. Results

3.1. *Characterization of PLGA–PEG–PLGA triblock copolymers*

A typical spectrum of PLGA–PEG–PLGA copolymer is shown in Fig. 1. The characteristic signals appearing at 5.2, 4.8, 3.6 and 1.5 ppm are assigned to the methine hydrogen of the DL-lactide units, methylene hydrogen of the glycolide units, the methylene hydrogen of the PEG and the methyl hydrogen of the DL-lactide units, respectively. The molecular weights, DL-lactide/glycolide molar ratio and polydispersity indexes of the copolymers are shown in Table 1.

3.2. *Gelation temperature of PLGA–PEG–PLGA copolymer solutions*

A typical phase diagram illustrating the gelation behavior of aqueous solutions of PLGA–PEG–PLGA(6/1) copolymer is shown in Fig. 2. The gelation temperature of the copolymers at various concentrations is shown in Fig. 3. Increasing the copolymer (6/1, 10/1, 15/1) concentrations from 15% (w/w) to 25% (w/w), the gelation temperature decrease from 36.2 to 31.2 °C, 35.4 to 30.2 °C and 34.5 to 29.5 °C, respectively. The gelation temperature also decreased with the increasing DL-lactide/glycolide molar ratio from 6/1 to 15/1. At all concentration levels, there was a significant difference ($P < 0.05$) in gelation temperatures among the three copolymers.

3.3. *In vitro erosion of the hydrogels*

Fig. 4 shows the effect of DL-lactide/glycolide molar ratio on the erosion of the copolymers. The

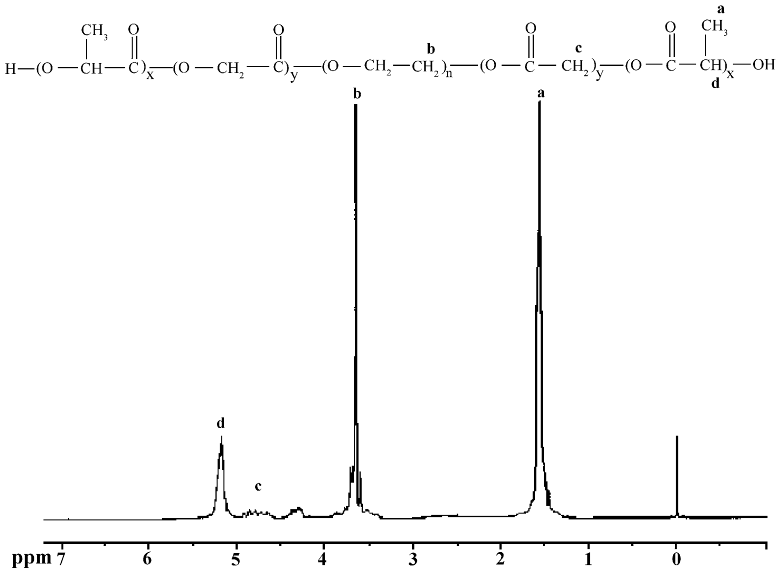

Fig. 1. The ^{1}H NMR spectrum of PLGA–PEG–PLGA(10/1) copolymer.

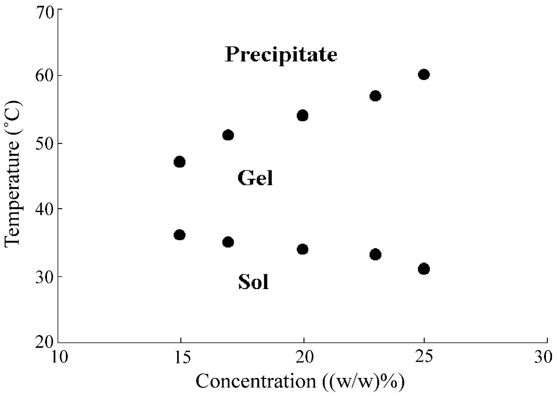

Fig. 2. Phase diagram of PLGA–PEG–PLGA(6/1) copolymer aqueous solutions.

PLGA–PEG–PLGA(15/1) copolymer displayed significantly lower erosion amount from day 15 to day 22, compared with the PLGA–PEG–PLGA(6/1) copolymer.

3.4. In vitro drug release studies

The release profiles of 5-fluorouracil and indomethacin from the PLGA–PEG–PLGA (6/1, 10/1 and 15/1) copolymer-based hydrogels are shown in Figs. 5 and 6. There is no difference ($P > 0.05$) in release rate for the 5-fluorouracil and initial release stage of indomethacin. Significant differences ($P < 0.05$) were found in the indomethacin release at day 15 to day 22 when comparing the PLGA–PEG–PLGA(6/1) with PLGA–PEG–PLGA(15/1). The comparison of 5-fluorouracil and indomethacin release from the

Table 1
The molecular weights, compositions and polydispersity indexes of the copolymers

Copolymer	Molecular weight of copolymer		DL-Lactide/glycolide ratio[b]	Polydispesity index
	M_w[a]	M_n[a]		
PLGA–PEG–PLGA(6/1)	4842	3824	5.7	1.27
PLGA–PEG–PLGA(10/1)	4584	3555	9.0	1.29
PLGA–PEG–PLGA(15/1)	5084	4067	14.8	1.25

[a] Measured by GPC, relative to polystyrene standards.
[b] Determined by ^{1}H NMR.

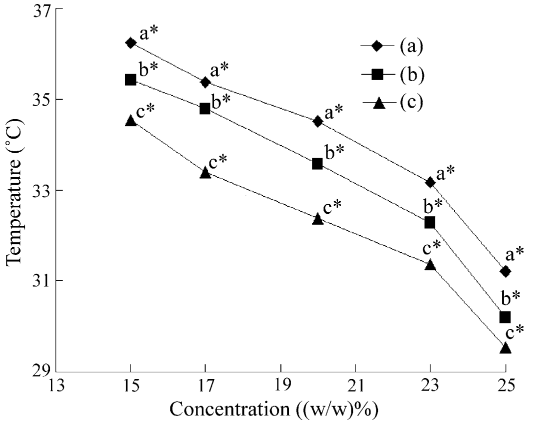

Fig. 3. Effect of DL-lactide/glycolide molar ratio on gelation temperature of the copolymers: (a) PLGA–PEG–PLGA(6/1), (b) PLGA–PEG–PLGA(10/1) and (c) PLGA–PEG–PLGA(15/1). (a) $^{*}P < 0.05$, PLGA–PEG–PLGA(6/1) vs. PLGA–PEG–PLGA(10/1). (b) $^{*}P < 0.05$, PLGA–PEG–PLGA(10/1) vs. PLGA–PEG–PLGA (15/1). (c) $^{*}P < 0.05$, PLGA–PEG–PLGA(15/1) vs. PLGA–PEG–PLGA(6/1).

PLGA–PEG–PLGA(6/1) copolymer-based hydrogel and ReGel system with a 0.5% (w/w) drug loading are shown in Figs. 7 and 8. Both the concentration of the copolymers were 25% (w/w). The PLGA–PEG–PLGA copolymer-based hydrogel showed significantly lower drug release rate than ReGel during the most of release period.

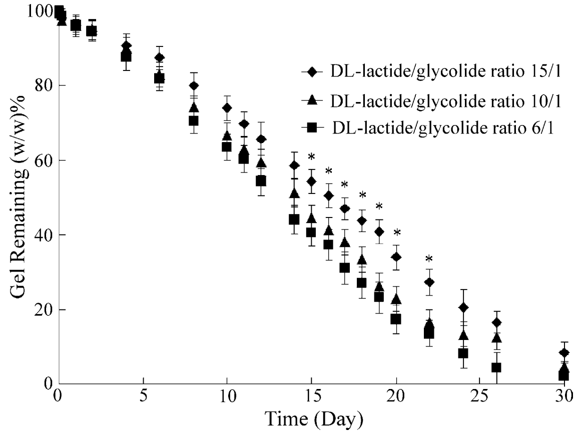

Fig. 4. Effect of DL-lactide/glycolide molar ratio on the erosion of copolymer at 37 °C. The copolymer concentration was fixed at 25% (w/w). The legend indicates the DL-lactide/glycolide molar ratio. Significance differences were found at indicated time points ($^{*}P < 0.05$, PLGA–PEG–PLGA(6/1) vs. PLGA–PEG–PLGA(15/1)).

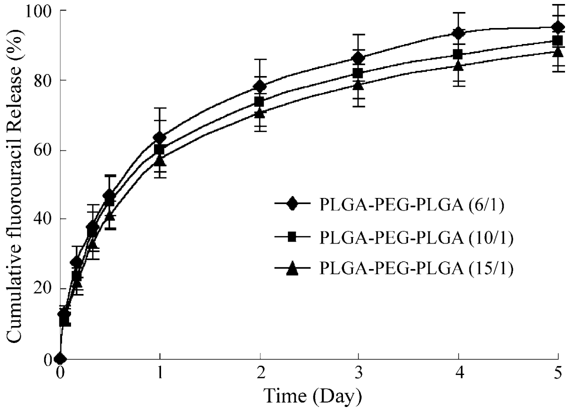

Fig. 5. Effect of DL-lactide/glycolide molar ratio on 5-fluorouracil release at 37 °C. The copolymer concentration was fixed at 25% (w/w) and the drug loading was 0.5% (w/w). The legend indicates the DL-lactide/glycolide molar ratio. Each point represents the mean ± S.D.; $n = 3$.

3.5. Drug release mechanism

The release data of 5-fluorouracil and initial release stage of indomethacin were fitted to both Eqs. (2) and (3), and the correlation coefficients are shown in Table 2. A typical diagram of cumulative indomethacin release during later release stage (10–30 days) plotted against cumulative erosion amount is shown in Fig. 9.

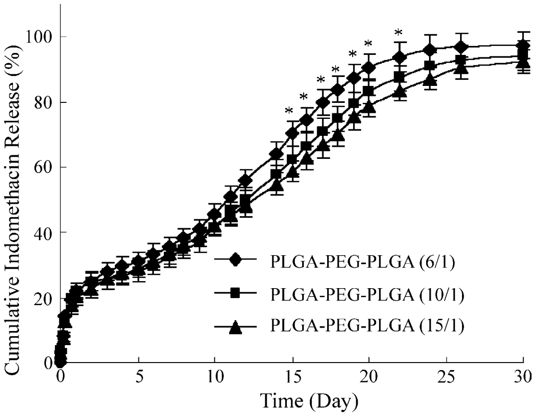

Fig. 6. Effect of DL-lactide/glycolide molar ratio on indomethacin release at 37 °C. The copolymer concentration was fixed at 25% (w/w) and the drug loading was 0.5% (w/w). The legend indicates the DL-lactide/glycolide molar ratio. Each point represents the mean ± S.D.; $n = 3$. Significance differences were found at indicated time points ($^{*}P < 0.05$, 6/1 vs.15/1).

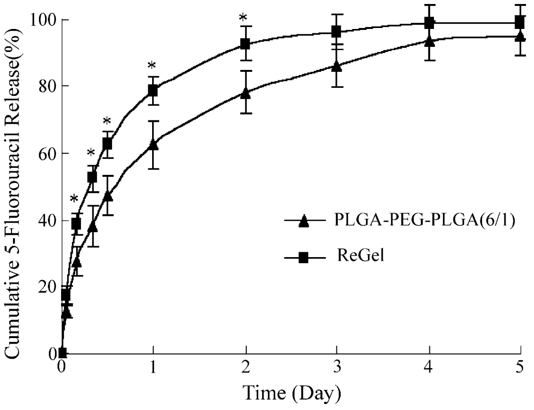

Fig. 7. 5-Fluorouracil release from PLGA–PEG–PLGA(6/1) and ReGel when the copolymer concentration was fixed at 25% (w/w). The drug loading is 0.5% (w/w). Each point represents the mean ± S.D.; $n = 3$. Significance differences were found at indicated time points ($^{*}P < 0.05$, PLGA–PEG–PLGA(6/1) vs. ReGel).

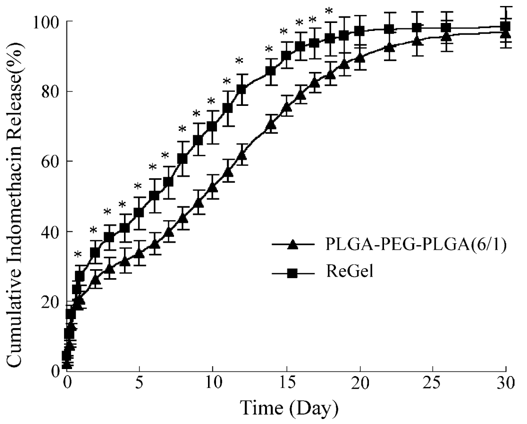

Fig. 8. Indomethacin release from PLGA–PEG–PLGA(6/1) and ReGel when the copolymer concentration was fixed at 25% (w/w). The drug loading is 0.5% (w/w). Each point represents the mean ± S.D.; $n = 3$. Significance differences were found at indicated time points ($^{*}P < 0.05$, PLGA–PEG–PLGA(6/1) vs. ReGel).

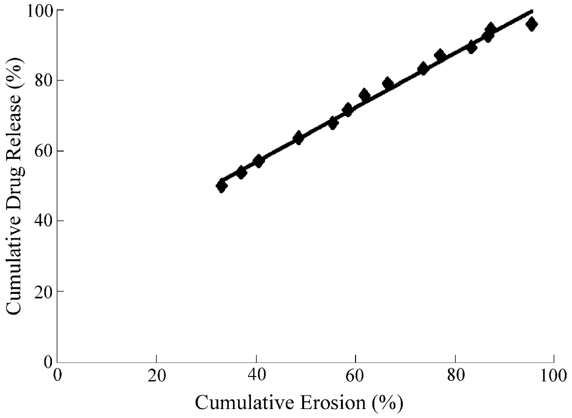

Fig. 9. Correlation between cumulative drug release (%) and erosion amount (%). The PLGA–PEG–PLGA(10/1) copolymer concentration was fixed at 25% (w/w).

4. Discussion

4.1. Effect of DL*-lactide/glycolide molar ratio on the hydrogel erosion and drug release*

Thermoreversible gelation in polymeric system is a well-known phenomenon. Hydrogel formation demands two seemingly contradictory kinds of behavior: interchain interaction must be strong to form junction points in the hydrogel network, yet at the same time the chain can not exclude solvent, or it will precipitate in water rather than form a hydrogel. Temperature-responsive PLGA–PEG–PLGA copolymers are a kind of block copolymers which are composed of hydrophobic PLGA segment function as formation of associative crosslinks and hydrophilic PEG segment which will permit the copolymer molecules stay in the solution. At lower temperatures, hydrogen bonding between hydrophilic PEG segments of

Table 2
Kinetic assessment of release data of 5-fluorouracil and indomethacin (0–9 days)

DL-Lactide/glycolide ratio	Eq. (2)				Eq. (3)			
	5-Fluorouracil		Indomethacin		5-Fluorouracil		Indomethacin	
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
6/1	0.638	0.997	0.254	0.996	0.525	0.978	0.0432	0.970
10/1	0.619	0.995	0.247	0.985	0.354	0.999	0.0444	0.994
15/1	0.581	0.999	0.248	0.995	0.298	0.999	0.0365	0.996

the copolymer chain and water molecules dominates in the aqueous solution, resulting in their dissolution in water. As the temperature increases, the hydrogen bonding becomes weaker, while hydrophobic forces among the hydrophobic PLGA segments strengthened leading to sol–gel transition. The hydrophobicity of the copolymer increases in the order PLGA–PEG–PLGA(6/1), PLGA–PEG–PLGA(10/1) and PLGA–PEG–PLGA(15/1) by increasing the molar ratio of DL-lactide/glycolide in the PLGA segment because DL-lactide moiety is more hydrophobic than glycolide. The copolymer with higher DL-lactide/glycolide molar ratio can form more stable hydrogels due to the stronger hydrophobic interactions among the copolymer molecules. This can be verified by the in vitro erosion test of the hydrogels. The rate of hydrogel erosion depends on the DL-lactide/glycolide molar ratio and decreases with the DL-lactide/glycolide molar ratio increased from 6/1 to 15/1 (see Fig. 4). The decrease of sol–gel transition temperature (see Fig. 3) also indicated that hydrophobic interactions increase among the copolymer molecules with the DL-lactide/glycolide molar ratio increase.

5-Fluorouracil and indomethacin were selected as the model of hydrophilic and hydrophobic drug to investigate the DL-lactide/glycolide molar ratio on the drug release from the copolymer-based hydrogel. It can be observed from Figs. 7 and 8 that the release of the 5-fluorouracil and initial release stage of indomethacin was similarly independent on the increasing of DL-lactide/glycolide molar ratio of the copolymer. However, during the later release stage of indomethacin, the release rate was significantly decreased with the DL-lactide/glycolide molar ratio raise from 6/1 to 15/1. This difference is related to the different drug release mechanism. Drug release from the hydrogels occurs by two principal mechanisms: (i) drug diffusion from the hydrogel during the initial release phase and (ii) release of drug by the erosion of the hydrogel matrix during the later release phase. The release data of 5-fluorouracil and indomethacin were fitted to both Eqs. (2) and (3). According to the models, a straight line is expected for each plot if drug release from the matrix is based on a diffusion mechanism. In both cases, good correlation coefficients were obtained suggesting that drug release was consistent with diffusion mechanism (Table 2). During later release phase (10–30 days) of indomethacin, straight lines with good correlation coefficients were obtained for the hydrogels with different DL-lactide/glycolide molar ratio when the cumulative release amount was plotted against cumulative erosion amount (see Fig. 9). This indicated that indomethacin release was controlled by hydrogel erosion from 10 to 30 days. The DL-lactide/glycolide molar ratio has less effect on the drug release dominated by diffusion, but significantly affected drug release dominated by hydrogel erosion. This may be due to the different region which the drugs distributed in the hydrogel. The hydrophobic indomethacin tends to partition into the hydrophobic PLGA domain of the hydrogel and only smaller amount partitions into the hydrophilic PEG domain, while the hydrophilic 5-fluorouracil tends to partition into the hydrophilic PEG domain (Fig. 10). Drug molecules distributed in the hydrophilic domain released by diffusing through the hydrophilic channels of the hydrogel which less changed with the variation of DL-lactide/glycolide molar ratio. The indomethacin molecules distributed in the hydrophobic PLGA domain released by hydrogel erosion. As discussed above, the rate of hydrogel erosion is decreased with the DL-lactide/glycolide molar ratio increase. Significant decrease in erosion amount of the hydrogels was found between 15 and 22 days compared PLGA–PEG–PLGA(15/1) with PLGA–PEG–PLGA(6/1). Therefore, similar results were obtained from the in vitro release test of indomethacin (see Fig. 6).

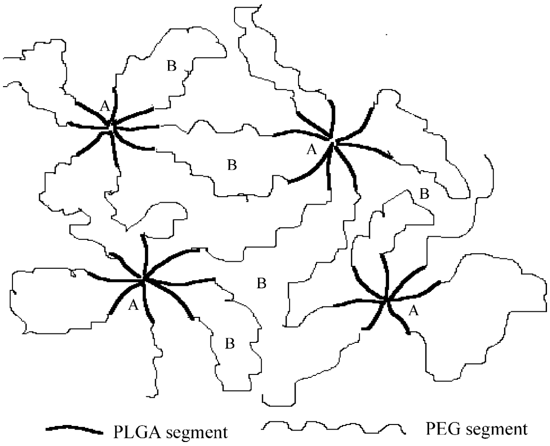

Fig. 10. A schematic diagram of the drug distributing in the hydrogel: (A) hydrophobic PLGA domain and (B) hydrophilic PEG domain.

4.2. The comparison of new synthesized copolymer with ReGel

For the temperature-responsive copolymers, it is important to precisely control the gelation temperature in designing a drug delivery system. Gelation temperature means the temperature below which the copolymer is soluble in water and above which the copolymer undergoes phase transition to form a water-insoluble hydrogel. If the gelation temperature is lower than room temperature, gelation will occur at room temperature leading to difficulty in manufacturing, handling and administering. If the gelation temperature is higher than body temperature, the formulation will maintain liquid at injection site resulting in drug leakage. The gelation temperature of ReGel system is lower than room temperature which needs to be improved. In contrast to the ReGel, the gelation temperature of copolymers synthesized by us range from 29.5 to 36 °C which is more suitable for drug delivery than ReGel (see Fig. 3). Furthermore, the new synthesized copolymer can provided longer drug release period at the same concentration level as ReGel (see Figs. 7 and 8). Thus, the copolymers synthesized by us have the advantages over the ReGel.

4.3. The method of drug loading on the solubilization efficiency

An important property of micelle is the ability to increase the solubility of sparingly soluble substances. The hydrophobic drugs can be physically incorporated within the hydrophobic cores of polymeric micelles and solubilized in the copolymer solution. Indomethacin is hydrophobic drug and practically insoluble in water. The solubility of indomethacin is 4 μg/ml at 25 °C (Hammad and Muller, 1998). The method of drug loading into the PLGA–PEG–PLGA copolymer micelles showed a great effect on solubilization efficiency. The physical mixing of solid indomethacin with the copolymer solution (25%, w/w) can only increase the solubility to 2.13 mg/ml. According to the solvent evaporation method, the drug is soluble in the copolymer solution (25%, w/w) up to 13.7 mg/ml. The solubility in the copolymer solution enhances 3425-fold compared to that in water. The main difference between the two methods is the adding of the drug before the micelle formation (solvent evaporation method) or after the micelle formation (physical mixing method). Micelles made of nonionic surfactants are known to have an anisotropic water distribution within their structure: water concentration decrease from the surface towards the core of the micelle. Due to this anisotropy, such micelles demonstrate a polarity gradient from the highly hydrated surface to the hydrophobic core. The hydrated surface interfere the incorporation of the hydrophobic indomethacin molecules into the micelle core when the drug was mixed with the micelle solution. In the case of the solvent evaporation method, indomethacin molecules were well distributed in the copolymer and intimately contacted with the PLGA segment of the copolymer after the evaporation. The solubilization occurred simultaneously with the copolymer micelle formation. This maybe the cause that the solvent evaporation method showed higher solubilization efficiency.

5. Conclusions

The temperature-responsive PLGA–PEG–PLGA copolymers with the different DL-lactide/glycolide molar ratio from the ReGel were synthesized and found to have the advantages over the ReGel. The effect of DL-lactide/glycolide molar ratio on the drug release was found to relate to the drug release mechanism. It has less effect on drug release dominated by diffusion but significantly affected drug release dominated by hydrogel erosion. The copolymers can solubilize the poorly water-soluble indomethacin and drug loading method shows a great effect on solubilization efficiency.

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References

Bhardwaj, R., Blanchard, J., 1996. Controlled release delivery system for the α-MSH analog melanotan-I using Poloxamer 407. J. Pharm. Sci. 85, 915–919.

Brown, W., Schillen, K., Almgren, M., Bahadur, P., 1991. Micelle and gel formation in a poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblock co-polymer in water solution:

dynamic and static light scattering and oscillatory shear measurements. J. Phys. Chem. 95, 1850–1858.

Cha, Y., Choi, Y.K., Bae, Y.H., 1997. Thermosensitive biodegradable polymers based on poly(ether-ester) block copolymers. US Patent 5 702 717.

Fults, K.A., Johnston, T.P., 1990. Sustained release of urease from a Poloxamer gel matrix. J. Parenter. Sci. Technol. 44, 58–65.

Hammad, M.A., Muller, B.W., 1998. Factors affecting solubility and stability of indomethacin in mixed micelles. Pharmazie 53, 790–794.

Jeong, B., Bae, Y.H., Lee, D.S., Kim, S.W., 1997. Biodegradable block copolymers as injectable drug delivery system. Nature 388, 860–862.

Jeong, B., Choi, Y.K., Bae, Y.H., Zentner, G., Kim, S.W., 1999. New biodegradable polymers for injectable drug delivery systems. J. Control. Rel. 62, 109–114.

Johnston, T.P., Palmer, W.K., 1993. Mechanisms of Poloxamer 407 induced hypertriglyceridemia in the rat. Biochem. Pharmacol. 46, 1037–1042.

Johnston, T.P., Punjabi, M.A., Froelich, C.J., 1992. Sustained delivery of interleukin-2 from a Poloxamer 407 gel matrix following intraperitoneal injection in mice. Pharm. Res. 9, 425–434.

Katakam, M., Bell, L.N., Banga, A.K., 1995. Effect of surfactants on the physical stability of recombinant human growth hormone. J. Pharm. Sci. 84, 713–716.

Kim, Y.J., Choi, S., Koh, J.J., Lee, M., Ko, K.S., Kim, S.W., 2001. Controlled release of insulin from injectable biodegradable triblock copolymer. Pharm. Res. 18, 548–550.

Lavasanifar, A., Samuel, J., Kwon, G.S., 2001. Micelles self-assembled from poly(ethylene oxide)-block-poly(*N*-hexyl stearate L-aspartamide) by a solvent evaporation method: effect on the solubilization and haemolytic activity of amphotericin B. J. Control. Rel. 77, 155–160.

Manohar, K., William, R.R., Ajay, K.B., 1997. Controlled release of human growth hormone in rats following parenteral administration of Poloxamer gels. J. Control Rel. 49, 21–26.

Miyazaki, S., Ohkawa, Y., Takada, M., Attwood, D., 1992. Antitumor effect of Pluronic F-127 gel containing mitomycin C on sarcoma-180 ascites tumor in mice. Chem. Pharm. Bull. 40, 2224–2226.

Rathi, R., Zentner, G.M., 1999. Biodegradable low molecular weight triblock poly(lactide-co-glycolide) polyethylene glycol copolymers having reverse thermal gelation properties. US Patent 6 004 573.

Rathi, R., Zentner, G.M., Jeong, B., 2000. Biodegradable low molecular weight triblock poly(lactide-co-glycolide) polyethylene glycol copolymers having reverse thermal gelation properties. US Patent 6 117 949.

Wang, P.L., Johnston, T.P., 1995. Sustained-release interleukin-2 following intramuscular injection in rats. Int. J. Pharm. 113, 58–65.

Wout, Z.G.M., Johnston, T.P., Pec, E.A., Maggiore, J.A., Williams, R.H., Palicharla, P., 1992. Poloxamer 407 mediated changes in plasma cholesterol and triglycerides following intraperitoneal injection to rats. J. Parent. Sci. Technol. 46, 92–200.

Yong, C.S., Choi, J.S., Quan, Q.Z., Rhee, J.D., Kim, C.K., Lim, S.J., Kim, K.M., Oh, P.S., Choi, H.G., 2001. Effect of sodium chloride on the gelation temperature, gel strength and bioadhesive force of Poloxamer gels containing diclofenac sodium. Int. J. Pharm. 226, 195–205.

Zentner, G., Rathi, R., Shih, C., Mcrea, J.C., Seo, M.H., Oh, H., Rhee, B.G., Mestechy, J., Moldoveanu, Z., Morgan, M., Weitman, S., 2001. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. J. Control. Rel. 72, 203–215.