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Controlled Delivery of Growth-Hormone-Releasing Peptide 6 from the Poly(lactic-co-glycolic acid)–Poly(ethylene glycol)–Poly(lactic-co-glycolic acid) Copolymer and the Effect of a Growth-Hormone-Releasing Peptide 6–Copolymer Hydrogel on the Growth of Rex Rabbits

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ABSTRACT: Growth-hormone-releasing peptide 6 (GHRP-6) plays an important role in animal growth. However, there have been few studies focusing on the effect of GHRP-6 on animal growth through controlled release systems. We synthesized the poly(lactic-co-glycolic acid) (PLGA)–poly(ethylene glycol) (PEG)–PLGA copolymer to investigate its controlled released effect on GHRP-6 *in vitro* and to study the effect of a GHRP-6–copolymer hydrogel on the growth of rex rabbits. The copolymer was synthesized with ring-opening copolymerization and characterized by ^{1}H-NMR. The interaction between GHRP-6 and the copolymer was characterized by Fourier transform infrared spectroscopy and X-ray diffraction. The body weight, serum level of insulin-like growth factor 1 (IGF-1), and hair coat quality were studied in rex rabbits. The results show that hydrogen bonds formed between the N—H group in GHRP-6 and the C═O group in the copolymer. The release mechanism of GHRP-6 was a combination of a diffusion-controlled mechanism and an erosion-controlled mechanism in the copolymer. The serum level of IGF-1, hair coat quality, and body weight were all significantly higher in the GHRP-6–copolymer hydrogel group than in the other groups. These results indicate that the copolymer effectively controlled the release of GHRP-6. In addition, the GHRP-6–copolymer hydrogel increased the synthesis of IGF-1 for a prolonged period and, thereby, increased the rex rabbits' growth and hair coat quality. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40185.

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INTRODUCTION

Growth-hormone-releasing peptide 6 (GHRP-6) is a six-amino-acid peptide, and the amino acid sequence is His-D–Trp–Ala–Trp–D–Phe–Lys–NH_2.[1] Clinical studies have demonstrated that GHRP-6 can increase animal growth, regulate immune function, improve animal production, and so on.[2,3] The previous biological function of GHRP-6 was achieved through the stimulation of the synthesis and secretion of growth hormone (GH). Bowers[4] reported that the level of GH and body weight were significantly increased in the GHPR-6-treated rats. GHRP-6 has a wide range of applications in animal production. Shih et al.[5] demonstrated that [His[1], Lys[6]]–GHRP could significantly improve the growth rate, feed efficiency, and carcass quality of pigs by intramuscular injection. However, there are some problems limiting the use of GHRP-6 in practical production. The major drawback is that GHRP-6 has a short half-life and is hydrolyzed quickly by enzymes *in vivo*. In addition, conventional formulations require daily administration; this brings inconvenience to the feeder, increases the economic costs, and limits its application in animal production. Therefore, a sustained delivery system for GHRP-6 is needed to make it more efficient in the growth and production of animals.

In recent years, several intelligent hydrogels have been extensively investigated in biomedical applications, such as the delivery of peptides and proteins.[6] These hydrogels exhibited a sol–gel phase transition in response to external stimuli, such as

temperature, pH, electric field, ionic strength, or their combination.[7] The poly(lactic-co-glycolic acid) (PLGA)–poly(ethylene glycol) (PEG)–PLGA copolymer is a kind of temperature-sensitive hydrogel, which has been widely studied because of its thermogelation, biodegradability, and nontoxicity.[8] This hydrogel exists as a solution at lower temperature. Under these conditions, peptides, proteins, cells, and other biologically active substances can be dissolved or dispersed in the aqueous solution of the polymer. The aqueous solution represents the sol–gel transition when the temperature exceeds the gelling point. Therefore, the embedded drug can be released in a sustained manner from the gel. Furthermore, the release time can be maintained for several weeks; thereby, a long-term sustained release effect is achieved. Choi et al.[9] reported that one injection of glucagon-like peptide 1 (GLP-1) loaded by the PLGA–PEG–PLGA copolymer could control the release of GLP-1 for 2 weeks; thereby the blood glucose concentration was controlled in type 2 diabetic rats.

Rex rabbits are a kind of economical animal. The hair coat of rex rabbits has a high economic value. In this study, the PLGA–PEG–PLGA copolymer was prepared, and it was used for the release of GHRP-6. The interactions of GHRP-6 and the copolymer were characterized by Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD). The sustained release of GHRP-6 *in vitro* and the effect of the GHRP-6–copolymer on the growth of rex rabbits were studied.

EXPERIMENTAL

Materials

Polyethylene glycol (PEG, including PEG1000 and PEG1500) was purchased from Shanghai Shiruike Biotech Co., Ltd. Glycolide and DL-lactide were purchased from Changchun Sinobiomaterial Co., Ltd. GHRP-6 was purchased from China Peptides Co., Ltd. Stannous 2-ethylhexanoic (Alfa Aesar) was obtained from Tianjin Chemical Co., Ltd. A bicinchonininc acid (BCA) Method Protein Assay Kit was purchased from Beijing BioTeke Biotechnology Co., Ltd. A rabbit insulin-like growth factor 1 (IGF-1) enzyme linked immunosorbent assay (ELISA) kit was purchased from Shanghai Jianglai Biotech Co., Ltd. Rex Rabbits were supplied by the Experimental Animal Center of Jilin University. All other chemicals were analytical grade.

Synthesis of the PLGA–PEG–PLGA Copolymer

Ring-opening polymerization was used to synthesize the PLGA–PEG–PLGA copolymer.[10] PEG1500 and PEG1000 were added to a three-necked flask and stirred at 120°C for 2 h *in vacuo* to dry. The mass ratio of PEG1500 to PEG1000 was 1:1. The molar ratio of DL-lactide/glycolide was 6:1, and the mass ratio of PEG was adjusted to 30% w/w in the reaction system. Stannous 2-ethylhexanoate (0.2% w/w) was added as a catalyst and heated in a thermostated oil bath at 150°C for 6 h *in vacuo*. The product was cooled with water (4°C). After that, the copolymer was heated to 80°C to precipitate the copolymer and remove the unreacted monomers. The supernatant was poured to obtain the precipitated copolymer. The previous process was performed three times to purify the copolymer. The purified copolymer was dried *in vacuo* at room temperature until a constant weight was reached.

Characterization of the PLGA–PEG–PLGA Copolymer

^{1}H-NMR was used to determine the composition and structure of the copolymer. The sample was dissolved in deuterated chloroform ($CDCl_3$). The spectrum was recorded at 500 MHz with an NMR instrument (Bruker, AVANCE III 500, Switzerland) at 25°C.

The molecular weights [weight-average molecular weight (M_w) and number-average molecular weight (M_n)] and molecular weight distribution (M_w/M_n) of the copolymer were determined by gel permeation chromatography (GPC). GPC measurements were carried out on a GPC instrument (Polymer Laboratory, GPC-50, United Kingdom) at 35°C. Tetrahydrofuran was used as a solvent with a flow rate of 1.0 mL/min. Polystyrene was used as the calibration standard.

Phase-Transition Temperature of PLGA–PEG–PLGA

The gel temperature of different concentrations of copolymer solution (15, 20, 25, and 30%) was measured via the test tube inversion approach.[11] Two-milliliter vials containing 0.5 mL of copolymer solution were stored at 4°C for 24 h. Each sample was immersed in a water bath for 15 min with a temperature increment of 1°C per step and started at 4°C. The sample was regarded as a sol–gel transition by the inversion of the vial in the case of no visual flow within 30 s.

In Vitro Release of GHRP-6

The effect of different drug loadings on the drug-release rate was studied. Different masses of GHRP-6 (2, 5, and 10 mg) were dissolved in 1 mL of copolymer solution (20% w/w) at 4°C overnight. The copolymer solution containing different concentrations of GHRP-6 were poured into 15-mL test tubes and transferred into a 37°C water bath for 15 min to form gels completely. One milliliter of phosphate-buffered saline (PBS; pH = 7.4) containing 0.02% w/v NaN_3 were added to the gels as a release medium and shaken at 25 rpm at 37°C for 24 h. All of the release media were collected to determine the amount of GHRP-6 and replaced with the same volume of fresh buffer every 24 h. The amount of GHRP-6 in the released samples was determined by the BCA method, which is one of the most commonly used methods for the measurement of protein content. A copolymer hydrogel without GHRP-6 was used as a blank control. The GHRP-6 content in the released sample was obtained from the standard curve of absorbance.

The release data of GHRP-6 were evaluated by the Korsmyer–Peppas equation, which is a classical method for analyzing the mechanism of drug release.[12] The following equation describes the drug release from the polymer:

$$M_t/M_\infty = kt^n (n \leq 0.6)$$

where M_t is defined as the amount of drug released at time t, M_∞ is the mass of drug released as time t approaches infinity, k is a kinetic constant associated with the diffusion rate, and n is the released exponent.

Stability of GHRP-6

Circular dichroism (CD) spectra were used to determine the released GHRP-6 with a J-810 CD spectrophotometer (Jasco, Tokyo, Japan). CD signals were recorded from 300 to 200 nm at ambient temperature. Fresh release medium (PBS) was scanned

to eliminate the background interference in the same wavelength range.

Interaction of GHRP-6 and the Copolymer

The blends of the copolymer/GHRP-6 were prepared by the solvent method.[13] Briefly, 1 mL of copolymer solution (20% w/w) containing appropriate ratios of GHRP-6 (2, 5, and 10 mg) was prepared in water. The GHRP-6–copolymer solution was poured into a Petri dish and dried to a constant weight.

The spectra of the copolymer alone, the pure GHRP-6, and the GHRP-6–copolymer blend were measured by an FTIR spectrometer (Thermo, Nicolet 6700). The samples were mixed with KBr (in a ratio of 1:100 and pressed to form pellets). The FTIR spectra were recorded in the range 4000–400 cm^{-1} at ambient temperature.

The XRD of the copolymer alone, the pure GHRP-6, and the GHRP-6–copolymer blend were measured by a diffractometer (Bruker, D8 AVANCE, Germany). Patterns were obtained with a step width of $2\theta = 0.1°$ between 3 and 45° at ambient temperature.

Effect of the GHRP-6–Copolymer on the Growth of Rex Rabbits

The study protocol was approved by the Ethics Committee on the Use and Care of Animals at Jilin University (Changchun, China). Jirong rex rabbits, 7 weeks old and about 1.5–2.0 kg in body weight, were used in this study. Twenty-four rex rabbits were randomly divided into four groups. All of the rex rabbits had free access to water and standard pellet feed. The rabbits were treated with 1 mL of GHRP-6 hydrogel (2 mg/mL), 1 mL of GHRP-6 (2 mg/mL), blank hydrogel (1 mL), and saline (1 mL) by subcutaneous injection, respectively. All of the injections were filtered through 0.22-μm Millipore Express filter membranes before injection. The weight of the rabbits was measured, and blood samples were obtained from the ear vein in each group at designed times (days 7, 14, 21, and 28). The blood samples were centrifuged to obtain serum and stored at 20°C until they were assayed for IGF-1 concentration. The serum level of IGF-1 was analyzed by a commercially available ELISA kit with polyclonal antibodies recognizing IGF-I as the capture antibody and horseradish peroxidase-labeled polyclonal IGF-I antibodies for detection. The absorbance at 450 nm was measured in a microplate reader (Labsystems Multiskan MS, 352, Finland). All of the samples were assayed in duplicate.

Measurement of the Hair Coat Quality

At the designated time, the animals were slaughtered, and the skin tissue was fixed in 10% neutral buffered formalin for 48 h, dehydrated in a graded series of alcohols, and embedded in paraffin. Transverse sections (5 μm thick) were prepared with a rotatory microtome and mounted on glass slides; this was followed by counterstaining with hematoxylin and eosin (HE). The sections were observed under a light microscope to count the number of follicles. The wool length and hide thickness were determined by a vernier caliper. The skin area (S; cm^2) was calculated by the following equation (Bai et al.[14]):

$$S = W^{0.75}/(A + 0.00002W)$$

where $W^{0.75}$ is the metabolic weight, A is a constant that is equal to 0.28 in winter and 0.27 in summer, and W represents the body weight (g).

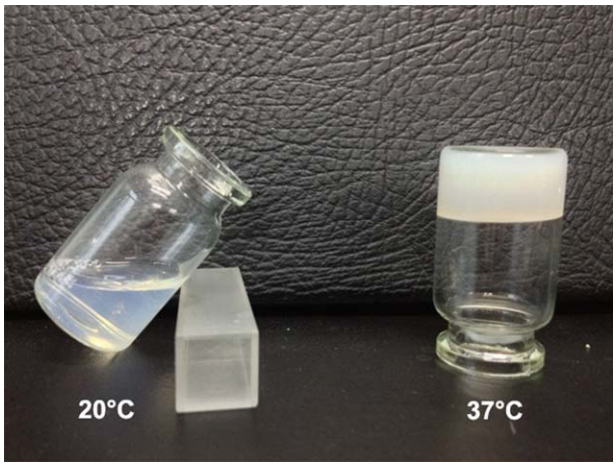

Figure 1. Aqueous solution of the copolymer at 20 and 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Statistical Analysis

Data for the effect of the GHRP-6–copolymer hydrogel in the rabbits were analyzed by a two-way analysis of variance (GraphPad Prism software). Values of $p < 0.05$ were considered as statistically significant, and values of $p < 0.01$ were considered highly significant. All data are presented as the mean plus or minus standard error of the mean.

RESULTS AND DISCUSSION

Synthesis and Characterization of the PLGA–PEG–PLGA Copolymers

Zentner et al.[15] reported that the DL-lactide/glycolide molar ratio was 3:1, and the molecular weight of PEG used for copolymerization was 1000 in ReGel, which is a trade name of the thermosensitive PLGA–PEG–PLGA copolymer. However, the gelation temperature was below room temperature, and this could bring trouble in the formulation, preparation, and administration. In this study, we successfully synthetized PLGA–PEG–PLGA by changing the molecular weight of PEG. The DL-lactide/glycolide molar ratio was 6:1, and the molecular weight of PEG used for copolymerization was 1000 and 1500 (mass ratio = 1:1). The aqueous solution (20% w/w) of the copolymer presented the solution state at 20°C and became a gel at 37°C (Figure 1). The phase diagram of PLGA–PEG–PLGA is shown in Figure 2. The phase-transition temperature of the aqueous solution was decreased from a sol to a gel and increased from a gel to a sol with increasing solution concentration. The characteristic signals of PLGA–PEG–PLGA in the ^{1}H-NMR spectrum appeared at 5.18 ppm (CH of lactide (LA), peak a), 4.81 ppm (CH_2 of glycolide (GA), peak b), 4.3 ppm (CH_2 of ethylene glycol, peak c), 3.66 ppm (CH_2 of ethylene glycol, peak d), and (e) 1.6 ppm (CH_3 of LA, peak e), respectively (Figure 3), which agreed with a previous report.[16] The molecular weight, molecular weight distribution, and DL-lactide/glycolide molar ratio of copolymer are listed in Table I. As shown in Table I, the DL-lactide/glycolide molar ratio of the copolymer showed good

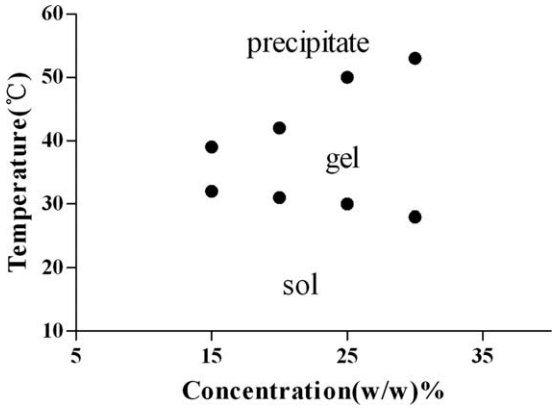

Figure 2. Phase diagram of an aqueous solution of the copolymer.

agreement with the feed ratio. The results of GPC indicated that the polydispersity of the copolymer was low enough to study its physical properties (Table I and Figure 4).

Interaction of GHRP-6 and the Copolymer

Drug–polymer interactions and the state of a drug in a polymer–matrix can be evaluated by XRD.[17] The XRD patterns of the copolymer alone, pure GHRP-6, and GHRP-6–copolymer blend are shown in Figure 5. Two peaks appeared in the diffraction patterns for pure GHRP-6 [Figure 5(a)]. The copolymer had only one broad peak in the XRD pattern, whereas the small peak at a 2θ scale of 5.5° disappeared in the GHRP-6–copolymer blends; this suggested that some interactions existed between GHRP-6 and the copolymer in the hydrogel, and GHRP-6 was either in an amorphous state or in the molecular dispersion in the copolymer.

The samples were also observed by FTIR spectroscopy to study the interactions between GHRP-6 and the copolymer. Many

Table I. Molecular Weight Compositions and Polydispersity Indices of the Copolymer Determined by GPC and ^{1}H-NMR

Copolymer	M_w[a]	M_n[a]	LA/GA[b]	M_w/M_n[a]
PLGA-PEG-PLGA	3422	2071	5.35	1.65

[a] Determined by GPC.
[b] Determined by ^{1}H-NMR

studies have used this method to investigate interactions in the drug–polymer blends. Chang et al.[18] studied the interactions of chitosan and gelatin gel with FTIR spectroscopy. In this study, we analyzed the spectra of the copolymer alone, pure GHRP-6, GHRP-6–copolymer blends, and the physical mixture of GHRP-6 and the copolymer with FTIR spectroscopy to assess the extent of drug–polymer interactions at the molecular level. The presence of shifts or changes in the spectra for the drug–polymer blends was an indication that interactions existed between the drug and the polymer. The FTIR spectra of the samples are shown in Figure 6. The pure copolymer exhibited a broad peak at 3504 cm^{-1} and a peak at 1758 cm^{-1} [Figure 6(e)], which were assigned to the stretching vibrations of O—H and C=O bonds, respectively. The spectra of all of the drug–polymer blends showed that the C=O group peak shifted to 1753 cm^{-1} [Figure 6(b–d)], and we also found that the absorption intensity of the C=O group significantly increased in the GHRP-6–copolymer blends. The FTIR spectra of the pure GHRP-6 showed N—H stretching at 3419 cm^{-1} [Figure 6(a)]. In the spectra of the GHRP-6–copolymer blends, the peak of the N—H stretching vibrations shifted to around 3328 cm^{-1} [Figure 6(b–d)]. The spectra of the C=O group and N—H group both shifted to lower wave numbers, and there was no significant

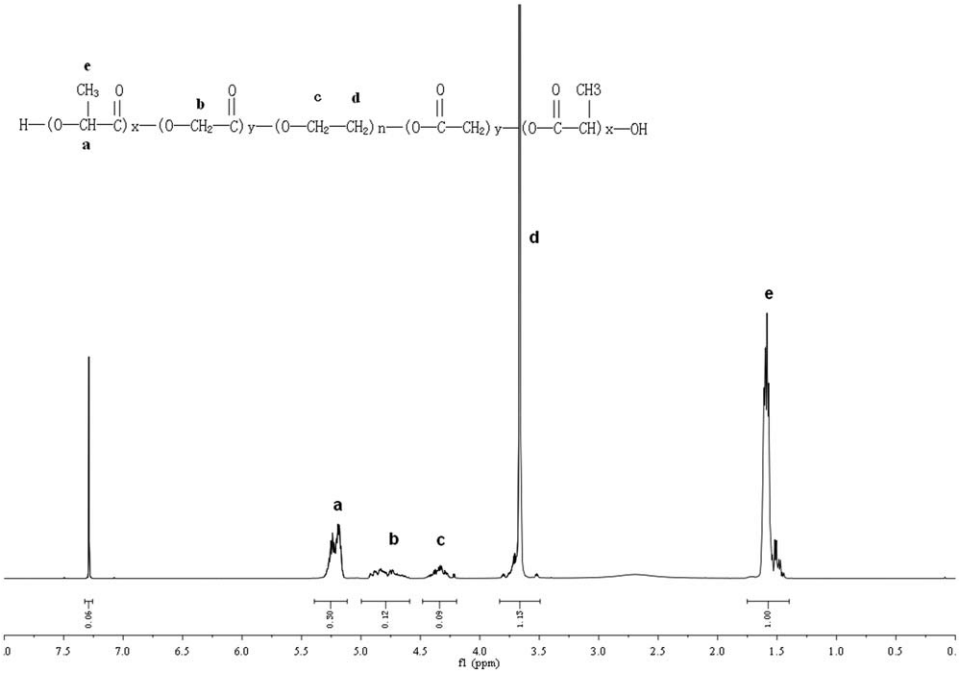

Figure 3. ^{1}H-NMR spectrum.

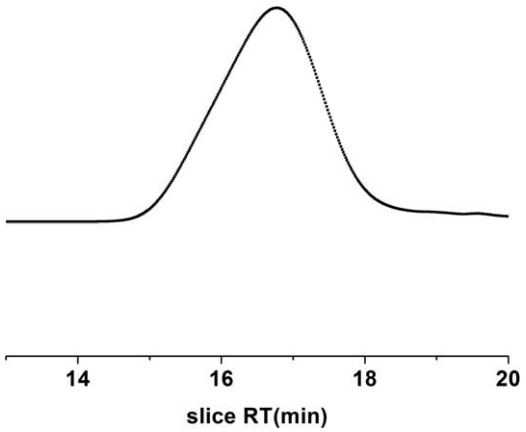

Figure 4. GPC trace. RT, retention time.

difference in the characteristic absorption peak between the pure copolymer and the physical mixture of GHRP-6 and the copolymer. These results indicate that hydrogen bonding possibly formed between the N—H group in GHRP-6 and the C=O group in the copolymer. The previous interactions may have directly slowed the release rate of drug and, thereby, depressed burst release. In addition, Qiao et al.[19] reported that hydrogen-bonding interactions can reduce the degradation rate of a drug–copolymer compared with a blank hydrogel.

Release Mechanism of GHRP-6

The drug-release mechanism from the polymer was described by several previous investigators.[20,21] The Korsmyer–Peppas equation is a classical method for analyzing the mechanism of drug release. This equation has two different meanings in two special cases of $n = 0.45$ (diffusion-controlled) and $n = 0.89$ (polymer-erosion-controlled). Values of n between 0.45 and 0.89 can be regarded as a combination of drug diffusion and polymer erosion. The two special values of n were valid for cylinder geometry.[22,23] In this study, GHRP-6 was used as a model

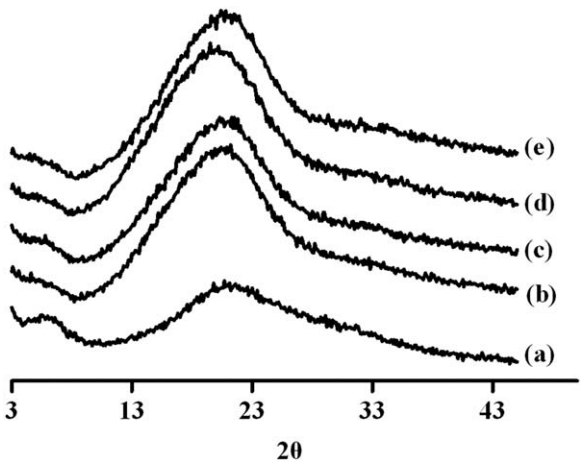

Figure 5. XRD patterns for GHRP-6, the PLGA–PEG–PLGA copolymer, and the various GHRP-6–copolymer blends: (a) pure GHRP-6, (b) GHRP-6 (10 mg)–copolymer blend, (c) GHRP-6 (5 mg)–copolymer blend, (d) GHRP-6 (2 mg)–copolymer blend, and (e) pure PLGA–PEG–PLGA copolymer.

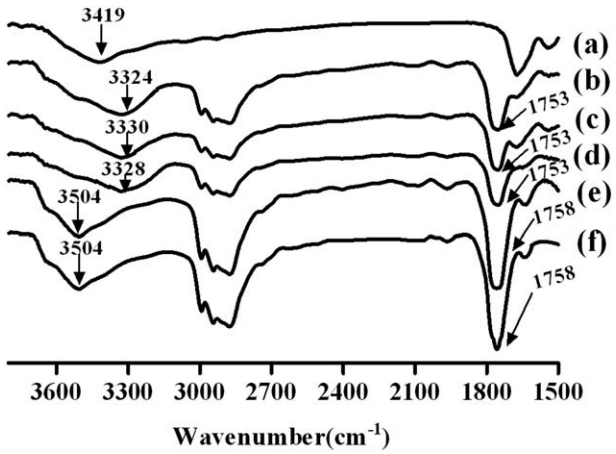

Figure 6. FTIR spectra of GHRP-6, the PLGA–PEG–PLGA copolymer, and the various GHRP-6–copolymer blends: (a) pure GHRP-6, (b) GHRP-6 (10 mg)–copolymer blend, (c) GHRP-6 (5 mg)–copolymer blend, (d) GHRP-6 (2 mg)–copolymer blend, (e) pure PLGA–PEG–PLGA copolymer, and (f) physical mixture of GHRP-6 and the copolymer.

peptide. Figure 7 shows the release of GHRP-6 from the hydrogels with different drug loadings. The release rate with a higher level of drug loading was faster than that with a lower level of drug loading. This trend was similar to previous results.[24] A high drug loading could decrease the copolymer density in the matrix. Therefore, water could move into the matrix, and the drug could be released from the matrix easily. The release of different GHRP-6 loadings (2, 5, and 10 mg) from the copolymer represented burst release in first 24 h, and the release percentages were 15.98 ± 1.24, 20.36 ± 1.56, and $23.39 \pm 2.57\%$, respectively. These results indicate that GHRP-6 burst release from the copolymer was mainly due to the release of surface-located drugs in the hydrogel. The release data of GHRP-6 were fitted to the Korsmeyer–Peppas equation and showed good correlations ($R^2 > 0.970$). Table II shows that the release exponents were 0.571, 0.477, and 0.481, which suggested that the release mechanism of GHRP-6 was a combination of GHRP-6 diffusion and copolymer erosion in the copolymer.

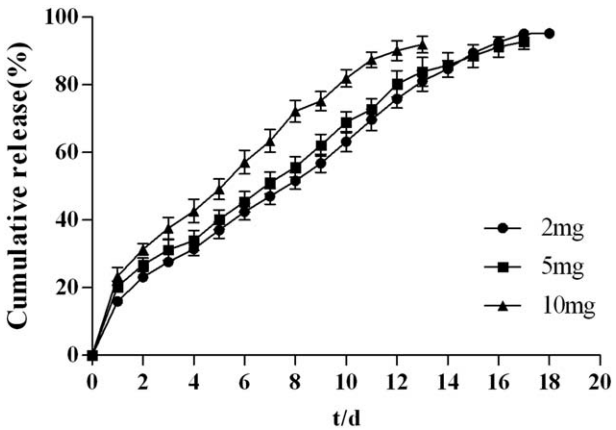

Figure 7. Cumulative release of various GHRP-6 samples from a 20% w/w concentration copolymer solution. Each point represents the mean plus or minus the standard error of the mean ($n = 3$). (t/d), time/day.

Table II. Kinetic Profile of GHRP-6 Release from the Hydrogel *In Vitro*

GHRP-6 loading (mg)	Slope	R^2
2	0.571	0.990
5	0.477	0.973
10	0.481	0.987

Stability of GHRP-6

The released GHRP-6 was determined by CD to assess the effects of the copolymer formulation on the stability of GHRP-6. As shown in Figure 8, the CD spectra of GHRP-6 released from the copolymer after days 7 and 17 showed no significant change compared to that of the freshly prepared native GHRP-6, which indicated that the released GHRP-6 preserved its secondary structure during the loading.

Effects of the GHRP-6–Copolymer Hydrogel on the Growth of Rex Rabbits

GHRP-6 is a growth-hormone-releasing peptide that plays an important role in the increase of animal growth. GHRP-6 is widely used in animal production, such as that of dairy cows, goats, calves, and pigs.[25,26] In addition, Croom et al.[27] reported that GHRP-6 could improve the milk production of dairy cows. In this study, rex rabbits were treated with the GHRP-6–copolymer hydrogel, GHRP-6 solution, saline, and the blank hydrogel, respectively, in which we investigated the effect of the GHRP-6–copolymer hydrogel on the growth of rex rabbits.

Studies demonstrated that GHRP-6 promoted the synthesis and secretion of GH in adenohypophysis. GH-activated Janus kinase 2/transcription factor signaled the transducer and activator of the transcription 5 signaling pathway to upregulate the synthesis and secretion of IGF-1 in hepatocytes.[28] The serum level of IGF-1 indicated the synthesis of GH. In this study, the concentration of serum IGF-1 was significantly higher in the GHRP-6 solution group than in other three groups in the first week [Figure 9(a)]. This indicated that GHRP-6 could significantly increase the synthesis of IGF-1. However, the concentration of serum IGF-1 was higher in the GHRP-6 copolymer hydrogel group than in the other three groups during the last 3 weeks. These results demonstrate that the hydrogel could control the release of GHRP-6, which maintained the serum of IGF-1 in a high level. IGF-1 plays an important role in animal growth, fetal development, and bone growth. Figure 9(b) shows that the average daily gain of the rex rabbits in the GHRP-6 solution group was higher than that in the GHRP-6 copolymer hydrogel and saline groups in the first week and increased by 12.15 and 64.09%, respectively. However, in the last 3 weeks, the average daily gain of the rex rabbits in the GHRP-6 copolymer hydrogel group was higher than that of the GHRP-6 group, and there was no significant difference between the GHRP-6 group and the saline group. The average cumulative weight gain of the rex rabbits in the GHRP-6 hydrogel group was significantly higher than that of the other three groups at 28 days [Figure 9(c)]. These results indicate that the GHRP-6 hydrogel effectively controlled the release of GHRP-6 and thereby improved the growth of the rex rabbits significantly.

Philpott et al.[29] reported that IGF-1 is an important physiological regulator in hair growth and the hair growth cycle. The evaluation parameters of hair coat quality are the follicle density, wool length, skin thickness, and skin area. In this study, the GHRP-6 hydrogel treated rex rabbits showed a high-quality hair

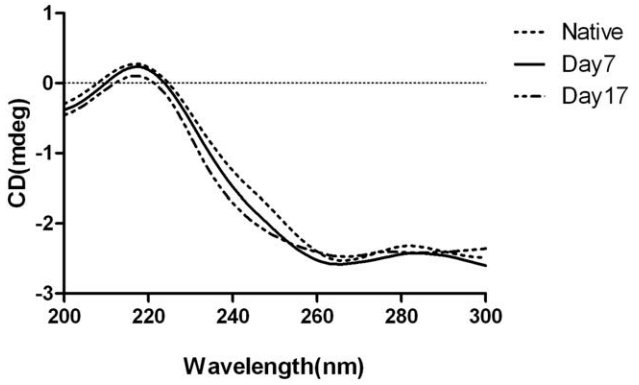

Figure 8. CD spectra of the GHRP-6 samples (5 mg/mL) released from a 20% w/w copolymer hydrogel. *Native* refers to a freshly prepared GHRP-6 solution in PBS.

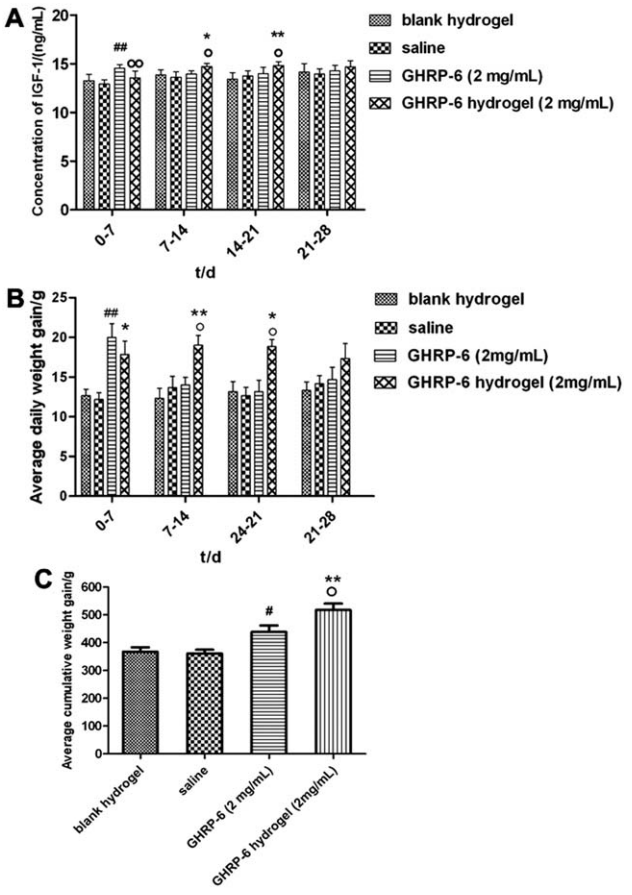

Figure 9. (A) Serum concentrations of IGF-1, (B) average daily weight gains, and (C) average cumulative weight gains in rex rabbits. #$p < 0.05$ and ##$p < 0.01$ for the GHRP-6 group versus the saline group; *$p < 0.05$ and **$p < 0.01$ for the GHRP-6 hydrogel group versus the blank hydrogel group; and °$p < 0.05$ and °°$p < 0.01$ for the GHRP-6 hydrogel group versus the GHRP-6 group. (t/d), time/day.

Table III. Wool Length, Skin Area, Skin Thickness, and Follicle Density of the Rex Rabbits

	Group			
Mark	Blank hydrogel	Saline	GHRP-6 (2 mg/mL)	GHRP-6 hydrogel (2 mg/mL)
Wool length (cm)	1.969 ± 0.057	1.974 ± 0.044	2.010 ± 0.064	2.070 ± 0.0112
Skin area (cm^2)	983.4 ± 34.13	989.0 ± 19.73	1010 ± 23.10	1048 ± 25.53[a]
Skin thickness (mm)	1.479 ± 0.11	1.488 ± 0.09	1.584 ± 0.09	1.608 ± 0.11
Follicle density (number/cm^2)	20,382 ± 945.7	20,760 ± 889.7	24,534 ± 1148	27,302 ± 1775[b,c]

[a] $p < 0.05$ for the GHRP-6 hydrogel group versus the blank hydrogel group.
[b] $p < 0.05$ for the GHRP-6 hydrogel group versus the saline group.
[c] $p < 0.01$ for the GHRP-6 hydrogel group versus the blank hydrogel group.

coat. The wool length and skin thickness were higher those in the GHRP-6 hydrogel group than in the other three groups, but there was no significant difference. However, the follicle density of the GHRP-6 hydrogel group was significantly higher than those of the hydrogel and saline groups. The skin area of the GHRP-6 hydrogel group was significantly higher than that of the blank hydrogel group (Table III). Furthermore, the results of HE showed that the follicle density was the highest in the GHRP-6 copolymer hydrogel group (Figure 10). Taken together, these results indicate that the hydrogel controlled the release of GHRP-6, which indirectly increased the synthesis of IGF-1 and, thereby, improved the rex rabbits' growth and hair coat quality. As shown by this study, the GHRP-6 copolymer hydrogel delivery system has great potential for applications in animal production, especially in fur animal production.

CONCLUSIONS

Hydrogen-bonding interactions existed between GHRP-6 and the PLGA–PEG–PLGA copolymer, as shown by FTIR spectros-

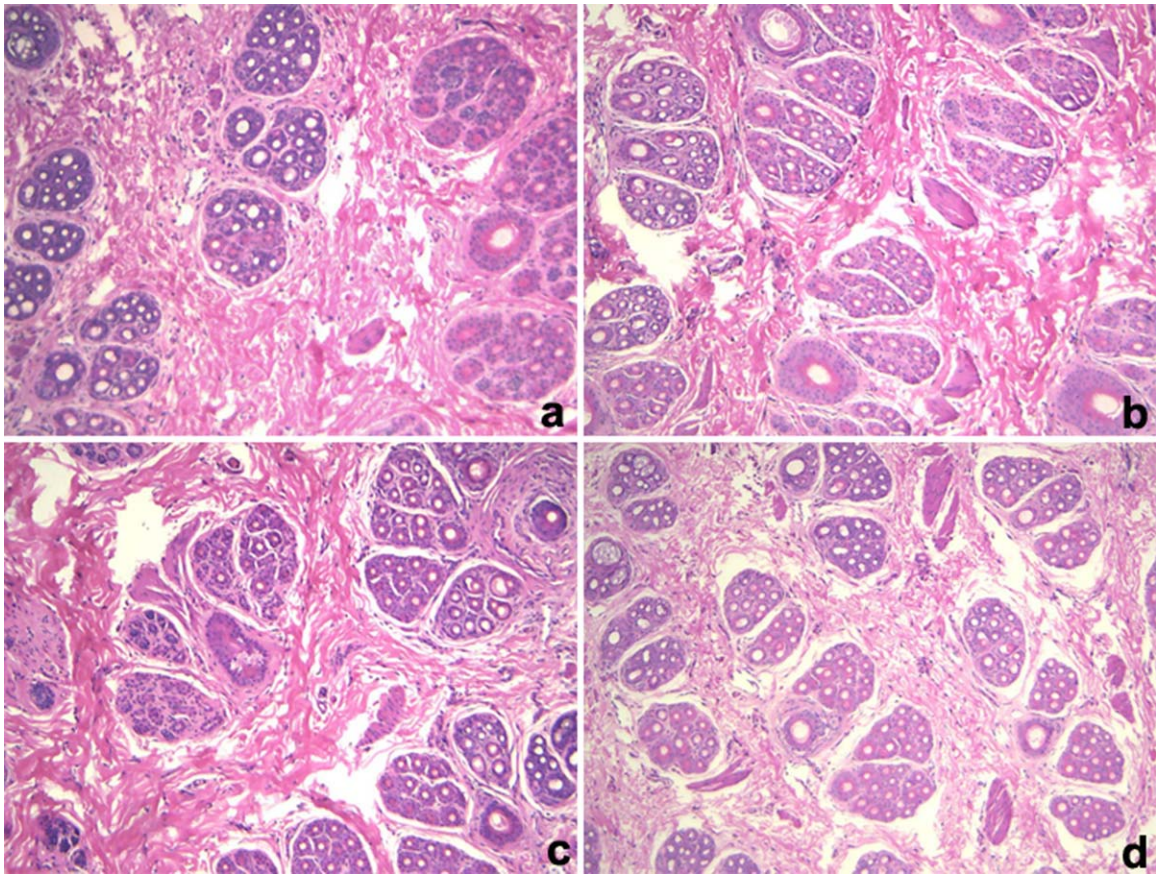

Figure 10. HE stains of the skin tissue: (a) saline (100×), (b) blank hydrogel (100×), (c) GHRP-6 (2 mg, 100×), and (d) GHRP-6 hydrogel (2 mg, 100×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

copy and XRD analysis. The copolymer effectively controlled the release of GHRP-6, and the release rate was associated with drug loading. In addition, the GHRP-6–copolymer hydrogel increased the synthesis of IGF-1 for a prolonged period and thereby increased the rex rabbits' growth and hair coat quality. However, further study is needed to investigate the burst release to retain the steady release of drugs and to improve the release effect through the study of the dose-effect relationship, the feed ratio of the copolymer, and the release mechanism.

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AUTHOR CONTRIBUTIONS

Songcai Liu, Linlin Hao, and Hao Yu supervised the research. Yuan Guan, Xibi Fang, and Dawei Zhang designed the research. Yuan Guan, Xinwei Li, Yunyun Cheng, Chao Lu, Dan Su, and Qiang Ma performed the experiments, and Yuan Guan and Xinwei Li wrote the article.

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