Carbohydrate Polymers 83 (2011) 1643–1651

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Biocompatibility and characteristics of injectable chitosan-based thermosensitive hydrogel for drug delivery

Hui Yun Zhou [a,b,*], Yan Ping Zhang [a], Wei Fen Zhang [b,c], Xi Guang Chen [b,**]

[a] Chemical Engineering & Pharmaceutics College, Henan University of Science and Technology, Luoyang 471003, PR China
[b] College of Marine Life Science, Ocean University of China, 5# Yushan Road, Qingdao 266003, PR China
[c] Department of Pharmaceutics, Weifang Medical College, Weifang 261042, PR China

ARTICLE INFO

Article history:
Received 4 October 2010
Accepted 8 October 2010
Available online 15 October 2010

Keywords:
CS-αβ-GP thermosensitive hydrogel
Hemolysis
Haematology and clinical biochemistry
Biocompatibility

ABSTRACT

CS-αβ-GP thermosensitive hydrogel was prepared with chitosan and αβ-GP which could be transited into gel at 37 °C within 10 min. The hemolysis rate of blank CS-αβ-GP gel and CS-αβ-GP gel loaded with ADR was 1.37% and 1.32%, respectively. The injected CS-αβ-GP gel did not produce any significant changes in the haematology of SD rats such as white blood cell, red blood cell, platelet and the volume of haemoglobin. In addition, the levels of serum alanine aminotransferase, blood ureic nitrogen and creatinine had no obvious changes in SD rats of different groups. So the CS-αβ-GP hydrogel had good blood compatibility and had no hepatotoxicity or renal toxicity to SD rats. Furthermore, CS-αβ-GP hydrogel had good tissue compatibility according to the inflammatory reaction in SD rats. CS-αβ-GP thermosensitive hydrogel was a promising delivery system which might be used as a long-acting drug delivery vehicle.

1. Introduction

Chitosan thermosensitive hydrogels prepared with different methods are of great interest in drug delivery (Bhattarai, Ramay, Gunn, Matsen, & Zhang, 2005; Kang & Song, 2008), cell encapsulation (Dang et al., 2006; Park et al., 2009), tissue engineering (Cho et al., 2004; Ma et al., 2010). Chitosan solutions containing β-glycerophosphate (β-GP) which has temperature-controlled solution–gel transition at a temperature close to 37 °C have recently been proposed as a suitable vehicle for the extravascular parenteral administration of drugs (Berger et al., 2005; Chenite et al., 2000; Cho, Heuzey, Bégin, & Carreau, 2005; Crompton et al., 2006; Ruel-Gariépy, Leclair, Hildgen, Gupta, & Leroux, 2002).

In addition, αβ-glycerophosphate (αβ-GP) is a mixture of β-glycerophosphate and α-glycerophosphate while α-glycerophosphate has linear chain structure and shows less steric hindrance than β-GP. Wu, Su, and Ma (2006) had reported a thermosensitive hydrogel of quaternized chitosan and αβ-GP and concluded that αβ-GP had better gelation capacity compared with β-GP. We had prepared chitosan thermosensitive hydrogel using chitosan and αβ-glycerophosphate (αβ-GP) (Zhou et al., 2008; Zhou, Chen, Kong, & Liu, 2009). The effect of different conditions on the characteristics, sol-to-gel transition and sustained release efficiency of hydrogel had been investigated. However, more study was needed to evaluate the biocompatibility and characteristics of chitosan-αβ-glycerophosphate (CS-αβ-GP) thermosensitive hydrogel in vivo as in situ delivery system.

In this paper, the CS-αβ-GP thermosensitive hydrogel was prepared with chitosan and αβ-GP by dissolving chitosan in acetic acid/sodium acetate buffer solution. Adriamycin (ADR) was chosen as a model drug, since it is a chemotherapy drug mainly used in the treatment of breast cancer, ovarian cancer, lung cancer and so on. The hemolysis, haematology, clinical biochemistry and biocompatibility in vivo were investigated after CS-αβ-GP thermosensitive hydrogel was injected intramuscularly into rats and transited into gel in situ at 37 °C.

2. Materials and methods

2.1. Materials

Chitosan, derived from crab shell, molecular weight: 1360 kDa; deacetylation degree 75.6%, prepared by the method of degradation with acetic acid. αβ-Glycerophosphate (αβ-GP), acetic acid glacial and sodium acetate were all chemical reagents of analytical grade provided by Sigma (St. Louis, MO). Adriamycin (ADR) was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd. (Taizhou, China).

* Corresponding author at: Chemical Engineering & Pharmaceutics College, Henan University of Science and Technology, 48# Xiyuan Road, Luoyang 471003, PR China. Tel.: +86 0379 64232193; fax: +86 0379 64232193.
** Corresponding author. Tel.: +86 0532 82032586; fax: +86 0532 82032586.
E-mail addresses: zhouhuiyun@hotmail.com (H.Y. Zhou), xgchen@ouc.edu.cn (X.G. Chen).

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doi:10.1016/j.carbpol.2010.10.022

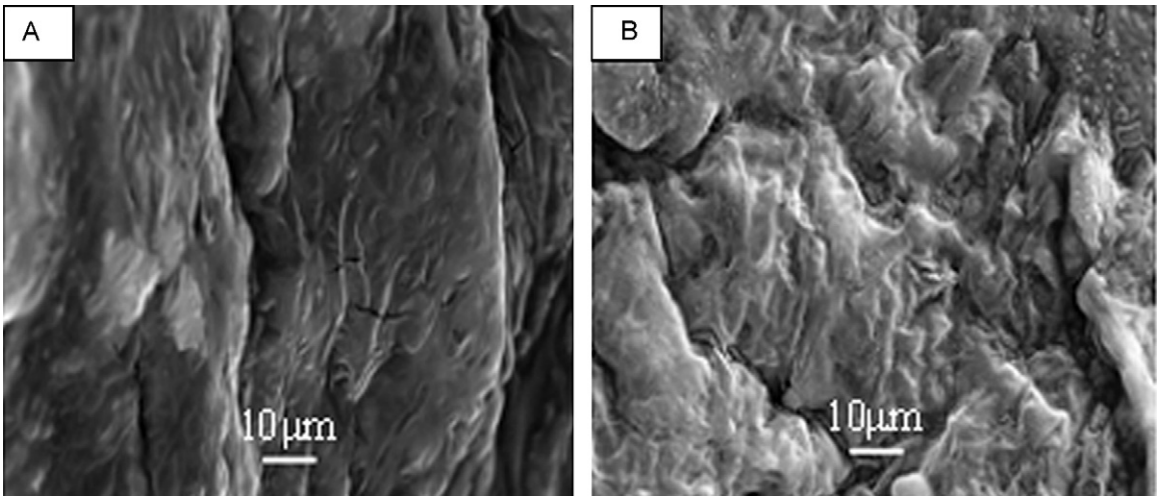

Fig. 1. SEM of CS-αβ-GP hydrogel: (A) CS-αβ-GP hydrogel without a model drug; (B) CS-αβ-GP hydrogel loaded with ADR.

Adult male Sprague–Dawley (SD) rats weighing 250–300 g were purchased from Qingdao Municipal Institute for Drug Control. The animal protocol was approved by Shandong Medical Laboratorial Animal Administration Committee. They were housed in a room with controlled temperature and humidity, and had free access to food and water. All animal experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 80-23) revised in 1996. All efforts were made to minimize the number of animals used and their suffering.

2.2. Preparation of CS-αβ-GP thermosensitive hydrogel

Chitosan (1.8 g) was added to 0.2 mol/l acetic acid/sodium acetate buffer (pH 4.6, 90 ml) with stirring until complete dissolution. Then the chitosan solution was chilled to 4 °C for 20 min. 50% (w/v) aqueous αβ-GP (10 ml) was prepared in distilled water and chilled along with the chitosan solution to 4 °C. Then the αβ-GP solution was added dropwise to the chitosan solution under stirring and the final chitosan-αβ-GP solution was mixed for another 20 min to form hydrogel. Finally, the CS-αβ-GP thermosensitive hydrogel was obtained and stored at 4 °C. Furthermore, CS-αβ-GP thermosensitive hydrogel loaded with adriamycin (ADR) was made with the same method. ADR was added to chitosan solution with agitating until it was dissolved thoroughly.

2.3. Sol-to-gel study of CS-αβ-GP thermosensitive hydrogel

A simple test tube inverting method was employed to determine the occurrence of sol-to-gel transition (Chung, Simmons, Gutowska, & Jeong, 2002). The sol phase was defined as flowing liquid and the gel phase as non-flowing gel when the hydrogel solution in the test tube was inverted. CS-αβ-GP thermosensitive hydrogel was added into a 5 ml tube to study sol-to-gel transition characteristics in a water bath of 37 ± 0.5 °C.

2.4. Scanning electron microscopy analysis

Samples (5 ml, in Cryogenic Vials) of hydrogel were incubated in a water bath of 37 ± 0.5 °C. When the hydrogel was transited into gel, the gel was frozen in liquid nitrogen and freeze-dried for 48 h (Christ Alpha 1-4 Freeze Dryer, Germany). The samples were coated with gold under vacuum and the surfaces were investigated with a scanning electron microscopy (KYKY2800B, KYKY Technology Development Ltd., China).

2.5. Hemolysis analysis

Hemolytic activity of the CS-αβ-GP thermosensitive hydrogel and materials used to prepare hydrogel were tested by direct contact methods, according to ISO 10 993-5. Whole rat blood was chosen to evaluate the hemolysis of specimen (Seibert, Shinohara, & Sano-Martins, 2003). Samples of CS-αβ-GP thermosensitive gel were prepared by the same method as used for microscopy analysis.

Anticoagulated rat blood (0.2 ml) was added to 10 ml of (i) 0.9% NaCl solution containing different specimens (0.25 g of chitosan, αβ-GP, CS-αβ-GP gel with or without ADR or 1 ml CS-αβ-GP hydrogel, respectively); (ii) physiological saline solution as negative control; and (iii) distilled water as positive control. Then the contents of the tubes were gently mixed and placed in water bath at 37 °C. After incubation for 1 h, the suspension was centrifuged at 1000 rpm for 10 min and absorbance of the supernatant of each tube were measured by ultraviolet spectroscopy (Tu-1800 UV–vis spectrophotometer, Beijing Purkinje General Instrument Co., Ltd.) at 545 nm. Triplicate samples were run. The rate of hemolysis was calculated according to the following equation:

$$\text{Rate of hemolysis } (\%) = \frac{\text{OD}_{\text{specimen}} - \text{OD}_{\text{negative}}}{\text{OD}_{\text{positive}} - \text{OD}_{\text{negative}}} \times 100$$

A mean hemolysis value from three test samples of 5% or less was considered acceptable.

2.6. Biocompatibility of CS-αβ-GP thermosensitive gel in vivo

2.6.1. Animal study

The in vivo biocompatibility and characteristics of CS-αβ-GP thermosensitive hydrogel were examined by implanting the gels in the skeletal muscle of SD rats via the intramuscular injection. The surgery thread was selected as negative control to evaluate the biocompatibility of hydrogels tested. SD rats were divided into four groups randomly as normal control group (without implantation), negative control group (implanted with surgery thread), experimental group I (injected with CS-αβ-GP thermosensitive hydrogel without ADR) and II (injected with CS-αβ-GP thermosensitive hydrogel loaded with ADR). CS-αβ-GP thermosensitive hydrogel were prepared as described formerly. The test specimens of hydrogel and surgery thread were sterilized by ultraviolet radiation for 30 min. Subsequently, the rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The CS-αβ-GP hydrogels (1 ml) were injected into the skeletal muscle of SD rats using a 18 G needle.

Table 1
Haematology parameters of rats in different time postoperatively (date shown were the mean ± SD, $n = 3$).

	Group	Normal control group	Negative control group	Experimental group I	Experimental group II
4 days	WBC ($\times 10^9$/l)	4.3 ± 0.9	3.7 ± 1.1	4.9 ± 0.7	4.4 ± 0.9
	RBC ($\times 10^{12}$/l)	5.05 ± 1.39	6.33 ± 0.97	5.89 ± 1.34	5.70 ± 1.62
	HGB (g/l)	100 ± 8	128 ± 13	115 ± 11	114 ± 9
	PLT ($\times 10^9$/l)	366 ± 121	222 ± 101	928 ± 114	664 ± 156
1 week	WBC ($\times 10^9$/l)	3.9 ± 1.1	4.2 ± 0.7	10.7 ± 0.9	10.2 ± 0.5
	RBC ($\times 10^{12}$/l)	5.65 ± 1.84	5.79 ± 1.57	5.35 ± 1.33	5.34 ± 0.84
	HGB (g/l)	110 ± 10	121 ± 13	100 ± 15	102 ± 8
	PLT ($\times 10^9$/l)	540 ± 220	305 ± 121	698 ± 150	834 ± 138
2 weeks	WBC ($\times 10^9$/l)	2.1 ± 0.8	4.2 ± 1.6	7.8 ± 0.7	7.6 ± 0.8
	RBC ($\times 10^{12}$/l)	5.74 ± 1.58	4.30 ± 1.76	5.14 ± 0.97	5.42 ± 0.79
	HGB (g/l)	122 ± 11	88 ± 9	103 ± 12	103 ± 14
	PLT ($\times 10^9$/l)	590 ± 246	326 ± 212	391 ± ±187	599 ± 136
4 weeks	WBC ($\times 10^9$/l)	8.9 ± 0.7	7.1 ± 0.7	9.1 ± 1.1	9.5 ± 0.8
	RBC ($\times 10^{12}$/l)	5.9 ± 1.91	6.1 ± 1.23	5.01 ± 1.67	5.87 ± 0.84
	HGB (g/l)	130 ± 13	123 ± 12	106 ± 11	115 ± 9
	PLT ($\times 10^9$/l)	319 ± 135	541 ± 115	600 ± 212	1225 ± 104
6 weeks	WBC ($\times 10^9$/l)	4.2 ± 1.2	4.4 ± 0.8	8.8 ± 0.7	9.3 ± 0.8
	RBC ($\times 10^{12}$/l)	5.49 ± 2.11	2.17 ± 1.19	5.19 ± 1.87	5.01 ± 2.38
	HGB (g/l)	105 ± 9	102 ± 10	92 ± 11	93 ± 13
	PLT ($\times 10^9$/l)	329 ± 164	206 ± 104	513 ± 167	364 ± 212
8 weeks	WBC ($\times 10^9$/l)	6.2 ± 1.4	3.6 ± 1.1	6.4 ± 0.9	6.6 ± 1.3
	RBC ($\times 10^{12}$/l)	5.16 ± 1.55	2.47 ± 1.13	5.43 ± 1.78	5.62 ± 2.13
	HGB (g/l)	117 ± 15	94 ± 9	100 ± 10	100 ± 11
	PLT ($\times 10^9$/l)	557 ± 187	259 ± 105	317 ± 137	587 ± 191
12 weeks	WBC ($\times 10^9$/l)	2.1 ± 0.7	4.1 ± 1.1	3.9 ± 1.7	3.5 ± 1.3
	RBC ($\times 10^{12}$/l)	3.88 ± 1.65	4.33 ± 1.13	5.46 ± 1.52	6.28 ± 1.70
	HGB (g/l)	83 ± 12	88 ± 9	109 ± 11	121 ± 13
	PLT ($\times 10^9$/l)	936 ± 123	418 ± 135	388 ± 214	476 ± 158

Each animal received two injections and the injected hydrogel was transited into gel in situ under the physiological temperature (near 37 °C). The surgery thread (10 cm) was implanted intramuscularly in the SD rats. The implantation was conducted by retracting both skin and muscles in the back of rats to form a cavity into which the specimen was inserted, approximately 1 cm left to the incision. The cavity and skin were then closed using 3-0 vicril surgery thread. Then the rats were supervised until complete recovery and then normal diet was resumed. At 4-day, 1, 2, 4, 6, 8 and 12 weeks postoperatively, 3 rats from each group were sacrificed and blood were collected via the jugular vein under anaesthesia and immediately transferred into prechilled MicrotainerR tubes. Heparinized blood samples were placed immediately on ice. Plasma was obtained by blood centrifugation at 3000 rpm for 20 min and then stored at −20 °C until used for analysis. The implanted surgery thread and gels along with their surrounding tissues were retrieved. The retrieved samples then were processed for histological and scanning electron microscopic examinations.

Table 2
Clinical biochemistry parameters of rats in different time postoperatively (date shown were the mean ± SD, $n = 3$).

	Group	Normal control group	Negative control group	Experimental group I	Experimental group II
4 days	ALT (U/l)	25.86 ± 8.24	50.07 ± 9.12	45.29 ± 9.56	53.67 ± 8.77
	Urea (mmol/l)	4.79 ± 1.34	6.07 ± 1.24	6.82 ± 1.33	7.60 ± 1.15
	Cr (mmol/l)	47.43 ± 9.87	84.31 ± 8.12	69.33 ± 7.57	74.22 ± 8.19
1 weeks	ALT (U/l)	37.69 ± 8.12	43.59 ± 8.83	35.37 ± 9.11	41.09 ± 7.67
	Urea (mmol/l)	3.84 ± 1.45	2.52 ± 1.24	6.17 ± 1.58	6.58 ± 1.78
	Cr (mmol/l)	57.72 ± 8.32	51.32 ± 7.88	60.44 ± 8.76	68.46 ± 9.03
2 weeks	ALT (U/l)	43.18 ± 7.99	45.74 ± 8.13	27.71 ± 8.23	36.91 ± 7.68
	Urea (mmol/l)	4.29 ± 1.16	5.06 ± 1.54	4.05 ± 1.33	5.04 ± 1.42
	Cr (mmol/l)	55.35 ± 7.18	55.56 ± 8.05	60.06 ± 8.67	58.42 ± 8.21
4 weeks	ALT (U/l)	64.66 ± 8.11	96.77 ± 9.87	50.05 ± 7.88	55.93 ± 8.05
	Urea (mmol/l)	5.58 ± 1.37	8.74 ± 1.22	6.24 ± 1.89	5.64 ± 1.66
	Cr (mmol/l)	72.09 ± 8.64	89.12 ± 8.63	73.84 ± 8.72	79.93 ± 9.04
6 weeks	ALT (U/l)	46.24 ± 9.12	33.31 ± 7.12	37.26 ± 7.67	43.17 ± 8.31
	Urea (mmol/l)	2.92 ± 1.06	3.08 ± 1.34	4.13 ± 1.63	4.81 ± 1.46
	Cr (mmol/l)	61.93 ± 7.86	60.95 ± 9.02	51.21 ± 8.57	58.11 ± 7.86
8 weeks	ALT (U/l)	39.09 ± 8.13	43.42 ± 8.01	34.05 ± 7.42	33.79 ± 8.07
	Urea (mmol/l)	2.88 ± 1.08	5.32 ± 1.25	7.59 ± 1.35	7.59 ± 1.64
	Cr (mmol/l)	25.05 ± 8.15	45.99 ± 7.68	45.89 ± 8.09	48.53 ± 8.19
12 weeks	ALT (U/l)	40.94 ± 9.06	45.55 ± 8.76	20.29 ± 7.64	29.69 ± 8.06
	Urea (mmol/l)	3.19 ± 1.14	2.96 ± 0.98	6.39 ± 1.87	6.71 ± 1.35
	Cr (mmol/l)	25.21 ± 7.18	31.22 ± 7.03	34.18 ± 8.66	35.59 ± 7.86

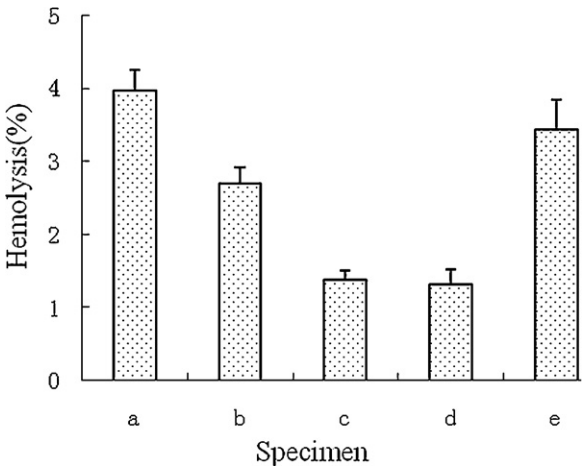

Fig. 2. Hemolysis rate of CS-αβ-GP gel and materials (date shown were the mean ± SD, $n = 3$). a: Chitosan; b: αβ-GP; c: CS-αβ-GP gel without ADR; d: CS-αβ-GP gel with ADR; e: CS-αβ-GP hydrogel without a model drug.

2.6.2. Haematology and clinical biochemistry analysis

The red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (HGB) and platelet (PLT) count of the whole blood were determined using the advia 120 automated haematology analyzer (Bayer Diagnostics, Newbury, Berkshire, UK). Serum was used for the estimation of alanine aminotransferase (ALT), blood ureic nitrogen (BUN) and creatinine (Cr) using automatic biochemical analyzer (Hitachi 7170A, Hitachi Ltd., Tokyo, Japan).

2.6.3. Histological examination

The samples used for the histological examination were fixed in 10% phosphate-buffered formaldehyde solution for at least 3 days. The fixed samples were embedded in paraffin, sectioned into a thickness of 5 μm, and then stained with haematoxylin and eosin (H&E). The stained sections of each test sample were examined using light microscopy (Nikon Microphoto-FXA) for tissue inflammatory reaction and photographed.

2.7. SEM examination of the implanted of CS-αβ-GP gel

The samples used for the SEM examination were first fixed with 2% glutaraldehyde in 0.1 mol/l of sodium cacodylate and then

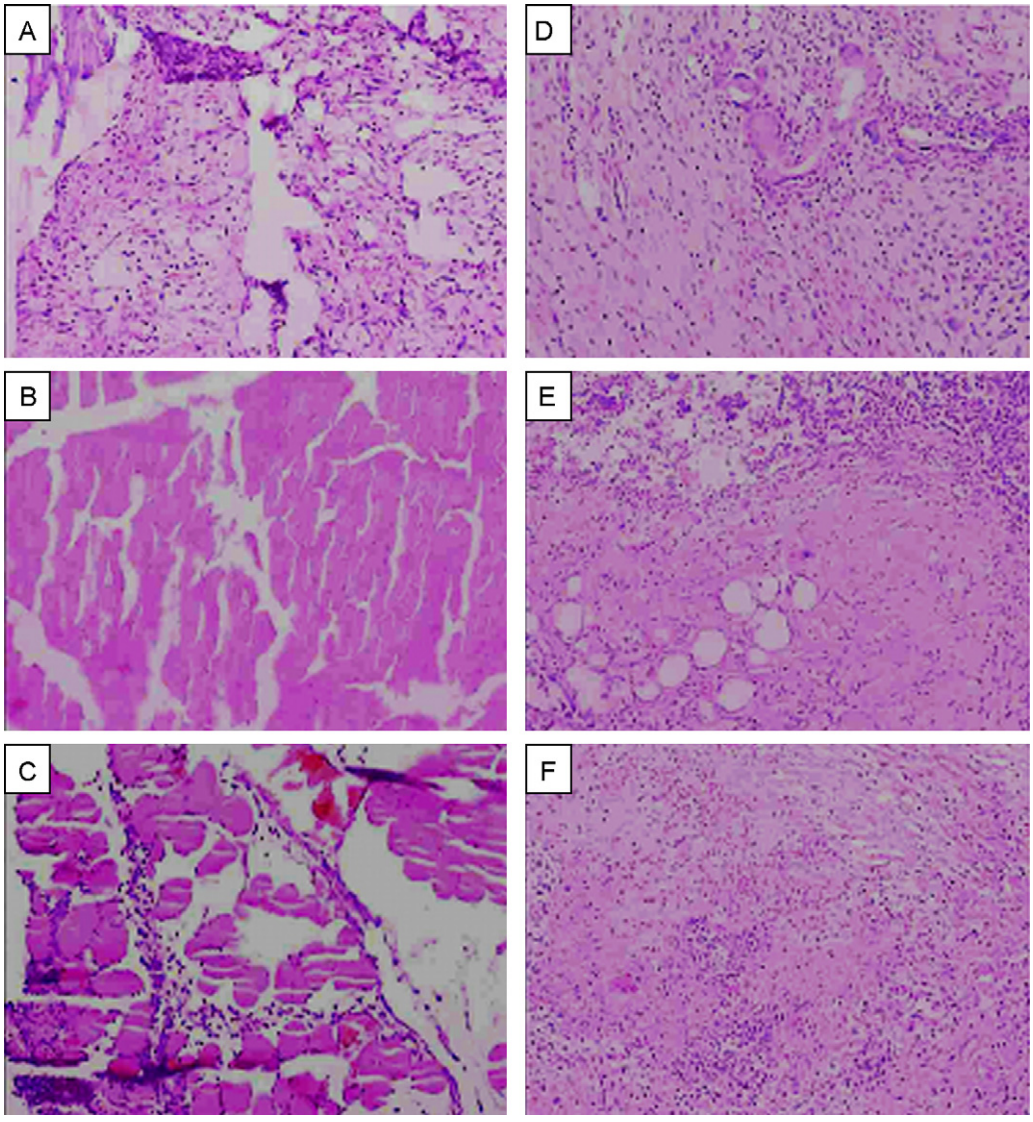

Fig. 3. Photomicrographs of the tissues implanted with (A) surgery thread; (B) CS-αβ-GP gel loaded with ADR; (C) blank CS-αβ-GP gel stained with haematoxylin and eosin retrieved at 4 days; (D) surgery thread; (E) CS-αβ-GP gel loaded with ADR; (F) blank CS-αβ-GP gel retrieved at 2 weeks (100×).

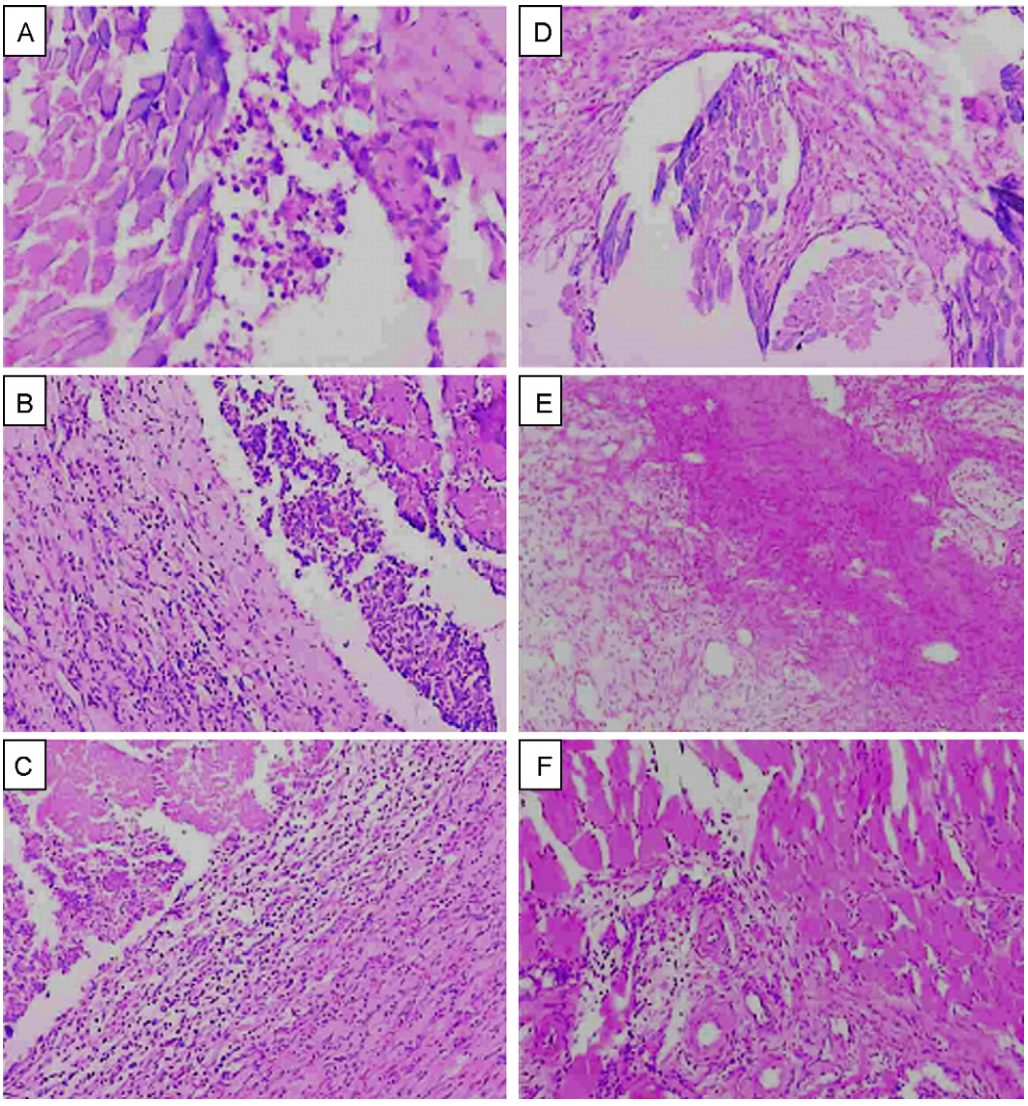

Fig. 4. Photomicrographs of the tissues implanted with (A) surgery thread; (B) CS-αβ-GP gel loaded with ADR; (C) blank CS-αβ-GP gel retrieved at 4 weeks; (D) surgery thread; (E) CS-αβ-GP gel loaded with ADR; (F) blank CS-αβ-GP gel retrieved at 6 weeks (100×).

post-fixed in 1% osmium tetroxide. Subsequently, the samples were dehydrated in a graded series of ethanol solutions, critical-point dried with carbon dioxide, and spattered with gold film. The examination was performed with a scanning electron microscope (KYKY2800B, KYKY Technology Development Ltd., Beijing, China).

2.8. Statistical analysis

The assays were performed in at least triplicate on separate occasions. The data collected in this study were expressed as the mean value ± standard deviation (SD).

3. Results and discussion

3.1. Characteristics of the CS-αβ-GP thermosensitive gel

The CS-αβ-GP hydrogel showed an apparent sol-to-gel transition at 37 °C, below which, the solutions were flowable viscous liquids and were injectable through a syringe. As the hydrogel was heated at 37 °C, it transformed into gel which was nonflowing within 10 min.

The SEM of hydrogels with or without ADR was shown in Fig. 1. It was shown that the appearance of hydrogel with or without a model drug were both compact and corrugated. In addition, there were little granules (small grain or pellet) in the appearance of gel loaded with ADR and the granules might be crystals of the added model drug (Fig. 1B).

3.2. Hemolysis of CS-αβ-GP thermosensitive gel

In vitro hemolysis testing is often used as an indicator of the injuries to the red blood cell membrane and considered to be a simple and reliable measurement for estimating blood biocompatibility of materials. Hemolysis index is regarded as safe when it is less than 5% (ISO document 10 993-5 1992). The method had been used to evaluate the biocompatibility of chitosan microspheres (Zhang, Zhou, Chen, Tang, & Zhang, 2009), films (Yang, Zhou, Chuo, Wang, & Yu, 2007), micelles (Zhang et al., 2008) and so on.

In this work, the hemolysis test of biomedical materials was used to measure hemocompatibility of CS-αβ-GP gel and the materials used. Under the conditions of this study, the tested materials and CS-αβ-GP gel were all safe and had no hemolytic activity for all the hemolysis value tested were not more than 5.0% (shown in Fig. 2). It could be seen that the hemolysis value of blank CS-αβ-GP gel and

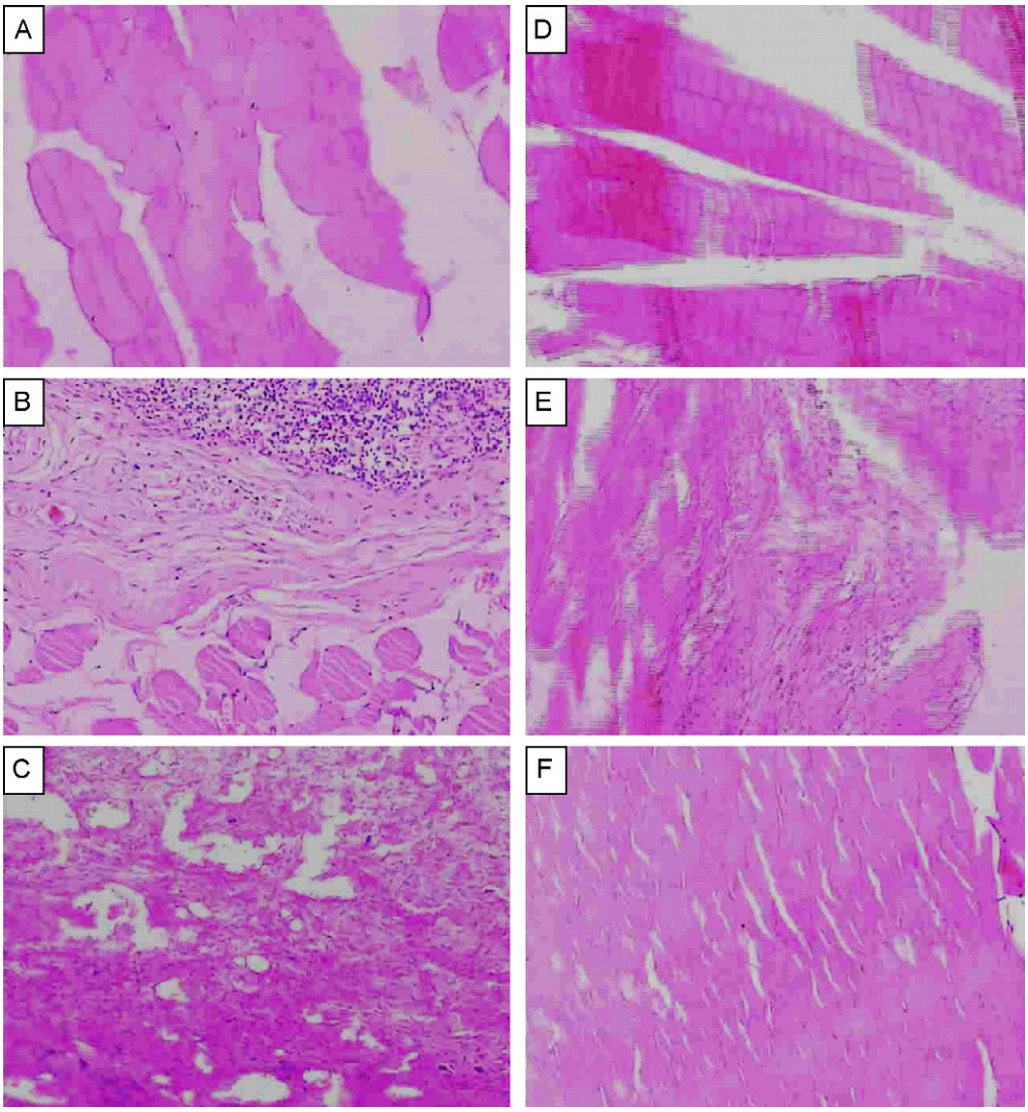

Fig. 5. Photomicrographs of the tissues implanted with (A) surgery thread; (B) CS-αβ-GP gel loaded with ADR; (C) blank CS-αβ-GP gel retrieved at 8 weeks; (D) surgery thread; (E) CS-αβ-GP gel loaded with ADR; (F) blank CS-αβ-GP gel retrieved at 12 weeks (100×).

CS-αβ-GP gel loaded with ADR was 1.37% and 1.32%, respectively, at the concentration of 25 mg/ml. It was obviously lower than that of chitosan and αβ-GP which was 3.92% and 2.68%, respectively, under the same conditions. There were broad range of molecular interactions between aqueous solutions of the cationic polyelectrolyte chitosan and the divalent anionic base of glycerol phosphate during the preparation of hydrogel and the sol–gel transition. The interaction decreased the cationic density of chitosan which might decrease the hemolysis rate of CS-αβ-GP gel. In addition, it was shown that the addition of ADR made no difference to the hemolysis rate of CS-αβ-GP gel. So it was indicated that the prepared CS-αβ-GP hydrogel had no hemolytic effect and could be used as a drug delivery system.

3.3. Haematology and clinical chemistry of CS-αβ-GP thermosensitive gel in vivo

Abnormal treatment-related values could represent changes pertaining to pharmacological and/or toxicological effects. These changes could be regarded as tissue morphology (detected by histopathological evaluation) and/or alterations in a series of in vivo analysed parameters. Dose-related changes were also of crucial importance. Among these parameters, clinical chemistry and haematology data are of great importance for determining effects induced by treatment. Reference range values could be obtained from control groups during each study or historical data from a number of control animal groups. This could provide information on the normal value in standard conditions for multiple points (animal housing, nutrition, beverage, hours of light per day, sex and age of animals, etc.). In this work, haematology and clinical chemistry of SD rats were studied to evaluate the safety of CS-αβ-GP hydrogel system. Meanwhile, haematology and clinical chemistry of SD rats without injection were studied as normal control and that of SD rats with surgery thread as negative control.

The count of WBC, RBC, PLT and the volume of HGB of SD rats were examined in 4-day, 1, 2, 4, 6, 8 and 12 weeks after injection or postoperatively and the haematologic results were shown in Table 1. The results were compared with that of control groups obtained in the same time after injection. It could be seen that the results were all in the normal range and that of blank CS-αβ-GP hydrogel or CS-αβ-GP hydrogel loaded with ADR had no obvious difference from that of normal control group or surgery thread group at different times postoperatively. So it was shown that the injection of CS-αβ-GP hydrogel did not produce any sig-

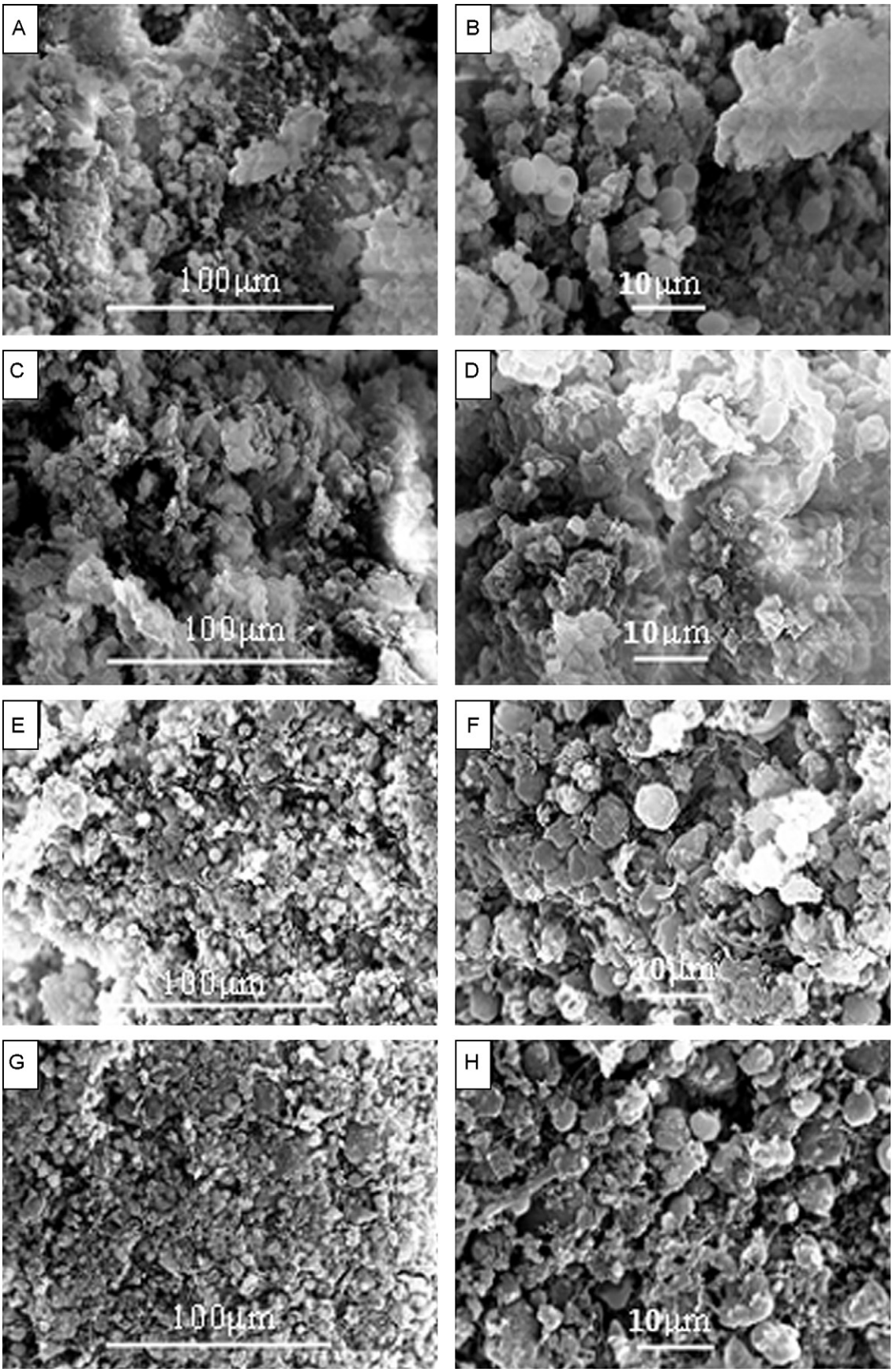

Fig. 6. SEM of implanted gel: (A and B) blank CS-αβ-GP gel; (C and D) CS-αβ-GP gel loaded with ADR retrieved at 2 weeks postoperatively; (E and F) blank CS-αβ-GP gel; (G and H) CS-αβ-GP gel loaded with ADR retrieved at 8 weeks postoperatively.

nificant changes in the haematology of SD rats such as WBC, RBC, PLT and HGB.

The results of ALT (U/l), BUN (mmol/l) and Cr (μmol/l) of SD rats injected with different CS-αβ-GP hydrogel and control groups at different time were shown in Table 2. It was shown that the levels of serum ALT, BUN and Cr had no significant changes in the experimental groups and normal control group or surgery thread group, which was in accordance with the good biocompatibility of chitosan reported previously (Souza, Zahedi, Allen, & Piquette-Miller, 2009; Zhang et al., 2009). So it could be considered that the injected CS-αβ-GP hydrogel had no hepatotoxicity or renal toxicity to SD rats.

3.4. Histological evaluation of CS-αβ-GP thermosensitive gel

The wounds were free from suppuration and necrosis after muscle implantation in all periods. Fig. 3 were the photomicrographs of the tissues implanted with surgery thread or injected with CS-αβ-GP hydrogel loaded with ADR or blank CS-αβ-GP hydrogel stained with H&E retrieved at 4 days or 2 weeks postoperatively. As shown in Fig. 3 (A–C retrieved at 4 days), there was a notable inflammatory reaction with neutrophils infiltration, a few lymphocytes and macrophages observed on the tissues among all studied groups. However, there was no significant difference from the three photomicrographs which indicated that the degree in inflammatory reaction observed in the experimental group I and II was almost the same as that implanted with surgery thread. At 2 weeks after injection or implantation, the degree of inflammation was much milder and neutrophils infiltration was significantly alleviated (seen in Fig. 3D–F).

At 4 weeks, the degree of inflammation for the tissue injected with CS-αβ-GP hydrogels or implanted with surgery thread declined significantly (shown in Fig. 4A–C). Furthermore, the fibrous structure was located at the interface surrounding the CS-αβ-GP hydrogel especially the blank CS-αβ-GP hydrogel (seen in Fig. 4C). Fig. 4D–F were the photomicrographs of the tissues implanted with surgery thread, injected with CS-αβ-GP hydrogel loaded with ADR or blank CS-αβ-GP hydrogel retrieved at 6 weeks. It was shown that the degree of inflammation of the tissues injected with CS-αβ-GP hydrogel was less than those retrieved at 2 weeks and 4 weeks postoperatively. For the group surgery thread, the degree of inflammation was also markedly reduced. At 8 and 12 weeks, the inflammatory reaction of the three groups was reduced sequentially and had almost disappeared at 12 weeks (shown in Fig. 5).

From the above comparison, it could be seen that the degree of inflammation was gradually reduced and disappeared in the end with time prolonged. Similar results had been described in previous reports (Mi, Tan, Liang, & Sung, 2002; Souza et al., 2009). The results suggested that CS-αβ-GP hydrogel with or without a model drug all had good tissue compatibility with the surrounding tissues and the degree of inflammation had no significant difference compared with that of SD rats implanted with surgery thread.

3.5. Morphology of the injected of CS-αβ-GP thermosensitive gel

After injection, the morphology of the gel changed progressively with time and finally disintegrated. At 2 weeks, CS-αβ-GP gel with or without a model drug had retained corrugated configuration. However the appearance of CS-αβ-GP hydrogel had become loose and the gel was surrounded with tissue cells (shown in Fig. 6A–D). At 8 weeks postoperatively, a looser structure was observed on the surface of gel and more little particles could be seen especially that of the CS-αβ-GP gel loaded with ADR, suggesting that degradation of gel started to occur (seen in Fig. 6E–H). The gel began to disintegrate at 12 weeks postoperatively (results not shown). The results indicated that the CS-αβ-GP hydrogel as a delivery system could survive a relatively long time in vivo and could be degraded gradually with time.

4. Conclusion

In this study, CS-αβ-GP thermosensitive hydrogel was prepared with chitosan (dissolved in the acetic acid/sodium acetate buffer solution) and αβ-GP. The hydrogel could be transited into gel at 37 °C within 10 min. The appearance of hydrogel with or without a model drug were both compact and corrugated. The hemolysis value of blank CS-αβ-GP gel and CS-αβ-GP gel loaded with ADR was 1.37% and 1.32%, respectively, at the concentration of 25 mg/ml. The results indicated that the prepared delivery system (CS-αβ-GP hydrogel) was safe and had no hemolytic effect. The results of haematology tested in vivo were all in the normal range and that of blank CS-αβ-GP hydrogel or CS-αβ-GP hydrogel loaded with ADR had no obvious difference from that of normal control group or surgery thread group at different postoperative times. The results of clinical chemistry of SD rats at different time indicated that the levels of serum ALT, BUN and Cr had no significant changes observed in experimental groups as compared to normal control group or surgery thread group. It was found that the degree of inflammation in all experimental rats was gradually reduced and disappeared with time. Meanwhile, the results of experimental groups had no significant difference from that of surgery thread Group. So CS-αβ-GP hydrogel with or without ADR all had good tissue compatibility. Furthermore, the morphology of the implanted gel showed that the delivery system could survive a relatively long time in vivo and could be degraded gradually with time lasting. These results suggested that CS-αβ-GP thermosensitive hydrogel was a promising delivery system which might be used as a long-acting intramuscularly implantable drug-delivery-vehicle via the intramuscular injection.

Acknowledgements

The authors are indebted to the financial support from National Natural Science Foundation of China (No. 30770582), the Natural Science Foundation of Shandong Province (No. Y2008F15), the Natural Science Foundation of Henan University of Science and Technology (No. 2007ZY020).

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