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An Injectable Hydrogel with or without Drugs for Prevention of Epidural Scar Adhesion after Laminectomy in Rats*

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Abstract Epidural scarring occurs inevitably in the defect after spinal laminectomy, and thus how to prevent or reduce it becomes a challenging topic. In the present study, an injectable hydrogel and its dexamethasone (DEX)-loaded hydrogel systems were adopted to prevent epidural scarring in a postlaminectomy rat model. The hydrogel system composed of poly(D,L-lactic acid-*co*-glycolic acid)-poly(ethylene glycol)-poly(D,L-lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers was a free-flowing sol at room temperature, and spontaneously turned into a semi-solid gel at body temperature. A lumbar 3 total laminectomy was performed on Sprague Dawley (SD) rats, and the efficacy of the injectable hydrogel with or without drugs in preventing epidural scar formation was evaluated *via* the gross anatomical observation and histological examination at one month post-surgery. The results demonstrate that the use of hydrogel alone reduced epidural scarring significantly, whereas the efficacy of the DEX-loaded hydrogels presented an irregular dose-dependency of drug and even the inappropriate drug doses resulted in the negative results. Therefore, the present study confirms that the PLGA-PEG-PLGA hydrogel holds potential as a barrier device to decrease peridural scarring, and reveals that the sustained delivery of the steroid hormone DEX to prevent surgery-related adhesions in the laminectomy defect is complex. Moreover, our *in vivo* studies also remind the researchers to pay attention to the irregular dose-dependency of the hormone drugs.

Keywords: Thermo-reversible hydrogel; Epidural scarring; Laminectomy; Steroid hormone.

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INTRODUCTION

Laminectomy is an operative process that is performed as a part of almost every kind of lumbar surgery because it can effectively accomplish nerve decompression[1]. Epidural scarring is a necessary process of wound repair and occurs after all spinal surgeries[2]. Dense, thick scar tissue adherent to the surgically exposed neural elements can lead to extradural compression or nerve root traction, thereby causing low back and leg pain: the so-called failed back surgery syndrome (FBSS)[3, 4]. What's more, the presence of tenacious scar tissues often makes subsequent spinal re-exposure procedures even more difficult and increases the risks of nerve root damage and

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dural tears[3, 4].

The development of surgical techniques and biomedical materials/devices that inhibits epidural scarring and thus prevents the adherence of the scar tissues to the dura mater and adjacent nerve roots is crucial for avoiding postlaminectomy complications. A variety of interposing materials including both natural matrices and synthetic materials have been used as barrier devices to prevent or reduce the formation of epidural fibrosis[5–7]. On the other hand, the abilities of some bioactive substances, such as immunosuppressive agent[8], anti-angiogenic medication[9] and anti-inflammatory drug[10], to prevent epidural adhesion have also been evaluated. For all these therapeutic schedules, only limited success has been achieved clinically, and none of them are extensively accepted with acknowledged results[11–13]. Therefore, new materials/devices that can effectively prevent peridural fibrosis are still desired.

Polymer hydrogels are three-dimensional networks which can absorb a large amount of water, and are widely used in many fields[14–18]. In the past decade, thermo-sensitive polymer hydrogels have attracted increasing attention because of their potential biomedical applications in sustained drug delivery and tissue engineering[19–24]. In general, these systems exhibit the sol states at low or room temperature and form the non-flowing gels as the temperature increases. Drugs and cells can be incorporated *via* simply mixing them with the polymer solution at low or room temperature and the corresponding formulation containing drugs or cells is easily injected into a specific body site using a conventional syringe. Due to contacting the body heat, the formulation spontaneously transforms into a semi-solid hydrogel, acting as a sustained drug delivery depot or a cell-growing matrix. Poly(ethylene glycol-*b*-propylene glycol-*b*-ethylene glycol) (PEG-PPG-PEG) and its derivatives[25, 26], PEG/polyester block copolymers[27–30], PEG/polypeptide block copolymers[31, 32], and poly(phosphazenes)[33, 34] are the representative thermo-sensitive polymers exhibiting the sol-gel transition in water with increasing temperature.

Among those mentioned above systems, the biodegradable and thermo-reversible hydrogels composed of block copolymers of PEG and aliphatic polyesters, such as poly(lactide-*co*-glycolide) (PLGA)[29, 35–37], poly(ε-caprolactone) (PCL)[24, 38], and poly(ε-caprolactone-*co*-lactide) (PCLA)[39, 40], have been extensively investigated due to the facile synthesis and good profile of safety. The sol-gel transition temperatures and degradation behavior of PEG/polyester hydrogels could be controlled *via* the block length, polyester composition, molecular weight distribution, and even end group[27, 28, 37, 39, 41–43]. To date, these hydrogels have been utilized as drug delivery carriers, tissue engineering scaffolds, barrier devices for post-surgical anti-adhesion and submucosal injection agents for endoscopic submucosal dissection[21, 44–51].

Dexamethasone (DEX), a famous steroid hormone, is widely used to treat chronic or active inflammation-related disease in clinic due to its good anti-inflammatory effect[52] and Fig. 1(a) shows the molecular structure of DEX. In this study, a thermo-reversible poly(D,L-lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-*co*-glycolic acid) (PLGA-PEG-PLGA) hydrogel was prepared, and the molecular structure of the copolymer is displayed in Fig. 1(b). On the one hand, the hydrogel alone was tried as a barrier device to prevent postlaminectomy epidural scarring and surgery-related adhesions. On the other hand, the pharmaceutical treatment of DEX was also adopted by loading this steroid hormone into the PLGA-PEG-PLGA hydrogel. An adult rat laminectomy model was employed to evaluate the *in vivo* efficacy and safety of the PLGA-PEG-PLGA hydrogel itself and its DEX-loaded hydrogel systems to prevent epidural adhesion, as schematically presented in Fig. 1(c). The corresponding results were examined and compared *via* gross anatomical observations, histological examinations and peel-off testing.

MATERIALS AND METHODS

Materials

PEG (1500), stannous octoate (purity of 95%) and dexamethasone (DEX, Bioreagent, purity ≥ 97%) were purchased from Sigma-Aldrich (USA). D,L-Lactide (LA) and glycolide (GA) were obtained from Purac (Netherlands). All other reagents were of reagent grade and used as obtained.

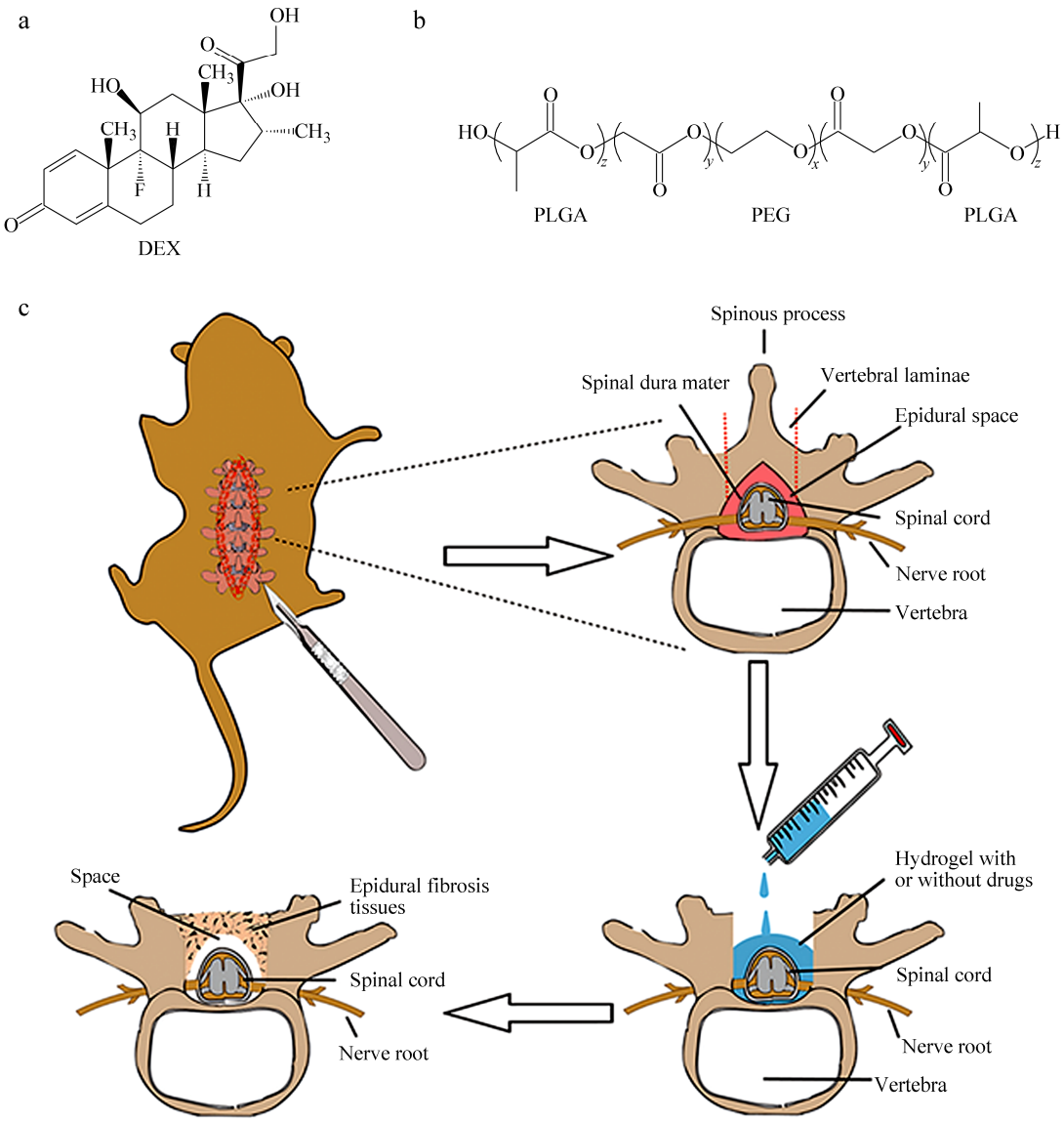


Fig. 1 (a) Molecular structure of DEX; (b) Molecular structure of PLGA-PEG-PLGA triblock copolymer; (c) Schematic diagram showing the applied procedure of the PLGA-PEG-PLGA hydrogel with or without drugs for prevention of peridural scar formation in a rat model of spine laminectomy
Four weeks after surgery, there was an available space between the dural sac and epidural fibrosis tissues under ideal situations.

Animals

A total of 46 male adult Sprague Dawley (SD) rats, each weighting between 300 g to 350 g, were provided by the department of laboratory animal science of Fudan University. The animals were housed under a 12 h light/dark cycle with appropriate humidity and temperature, and were free to access food and water. Thirty-five rats were selected to undergo a total L3 laminectomy. All experimental procedures were in accordance with the protocols approved by the Animal Care and Use Committee of Zhongshan Hospital, Fudan University.

Synthesis of PLGA-PEG-PLGA Triblock Copolymer

PLGA-PEG-PLGA triblock copolymers were synthesized *via* ring-opening copolymerization of LA and GA using PEG as the initiator. The detailed procedure was described in our previous publications[28, 53].

Physical-Chemical Characterization

The ^{1}H-NMR spectrum of the PLGA-PEG-PLGA triblock copolymer was recorded on a Bruker DMX500 NMR spectrometer at 500 MHz using $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard. Gel permeation chromatography (GPC, Agilent 1260) was carried out to determine the polymeric molecular weight (MW) and its MW distribution (M_w/M_n). Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1.0 mL/min at 35 °C. Monodispersed polystyrene (PS) standards were used to obtain the calibration curve.

Sol-Gel Transition

The sol-gel transition temperatures were determined *via* the vial inverting method with temperature increments of 1 K per step[53, 54]. Each sample with a given concentration was prepared *via* dissolving the polymer in normal saline (NS) solution. Next, 0.5 mL polymer solution was transferred to a 2 mL vial and stored at 4 °C for 12 h. Then, vials containing the samples were immersed in a water bath and maintained at each temperature for 10 min. Gel formation was determined in the case of no visual flow within 30 s after the vials were inverted by 180°.

Rheological Analysis

The PLGA-PEG-PLGA aqueous solutions (25 wt% in NS) containing DEX with three different concentrations (4 mg/mL, 2 mg/mL, and 1 mg/mL) were prepared by mixing the drugs with the polymer solutions under continuous stirring at 4 °C. The dynamic rheological properties of the PLGA-PEG-PLGA aqueous solutions with or without drugs were measured by a stress-controlled rheometer (Kinexus, Malvern) using a conical plate (diameter: 60 mm, conical angle: 1°, gap: 0.03 mm). 1.5 mL polymeric solution was transferred to the conical plate at 10 °C and the edge of conical plate was sealed with a small amount of low-viscosity silicone oil to minimize the evaporation of solvent. All rheological measurements were carried out at a single angular oscillation frequency of 10 rad/s and strain was kept within the linear viscoelastic range. Dynamic shear storage modulus G', loss modulus G'' and complex viscosity η^* ($\eta^* = (\sqrt{G'^2 + G''^2})/\omega$, ω: oscillation frequency) were recorded as a function of temperature. The heating rate was 0.5 K/min controlled by the plate temperature control semiconductor with a temperature resolution of 0.001 K.

Hemolysis Assay

0.1 mL of whole blood of White New Zealand rabbit was injected to 10 mL of the PLGA-PEG-PLGA solution in NS at the given concentrations. The samples were incubated in a water bath under 50 stoke/min at 37 °C for 1 h and then were centrifuged at 1000 r/min for 5 min. The concentration of hemoglobin in the supernatant was determined by UV absorption spectroscopy with absorbance at 540 nm. For comparison, pure NS solution was set as the negative control group and distilled water was set as the positive control group. The hemolysis rate of the triblock copolymer aqueous solution is calculated:

$$\text{Hemolysis (\%)} = \frac{D_s - D_{ns}}{D_{dw} - D_{ns}} \times 100\%$$

D_s is the average optical density of copolymer solution, and D_{ns} and D_{dw} refer to the average optical density of NS and distilled water.

In vitro Degradation

The PLGA-PEG-PLGA triblock copolymer aqueous solutions (25 wt%, 0.5 mL) were added into vials (inner diameter: 13 mm) and stored at 4 °C for 12 h to eliminate bubbles. The samples were incubated in a shaking bath with 50 stoke/min at 37 °C for 10 min to form hydrogels, and then 10 mL of phosphate buffered saline (PBS) solution (pH 7.4) was added. Buffer solution was replaced by a fresh one per 5 days to maintain the medium pH. At scheduled intervals, some samples were taken out of vials, and the remaining gels were lyophilized until constant weight. To eliminate the effect of salts in PBS, blanks containing the same volume of PBS solution were also lyophilized. For analysis of samples, measurements of GPC and weighting were performed. The pH change of buffer was also recorded with a pH meter at predetermined time points before the medium was substituted by a fresh one.

In vivo Degradation

The PLGA-PEG-PLGA aqueous solution (25 wt% in NS) was sterilized by the irradiation of Co-60 before use. Eleven SD rats were anesthetized and then 0.4 mL of the polymer aqueous solution was subcutaneously injected into the back of animal using a 1 mL syringe. Each animal simultaneously received two injections at the different sites of back. At each scheduled time point, one (30 min post-injection) or two animals (1, 2, 3, 4 and 5 weeks post-injection) were sacrificed. The remaining gels were carefully taken out from the injection sites and recorded using a digital camera. Weighting and MWs analysis of the remaining gels were performed.

In vitro Release of DEX

0.5 mL of the DEX-loaded PLGA-PEG-PLGA aqueous solution was injected into a 0.5 mL dialysis cassette (MWCO, 3500 Da, Thermo Scientific) and then placed in a weighing bottle at 37 °C for 10 min to form hydrogel. Next, 20 mL of PBS solution (pH 7.4) containing 0.02 wt% NaN_3 was added into each weighing bottle. The weighing bottles were then enclosed and incubated in a shaking bath with 50 stoke/min at 37 °C. At scheduled intervals, release medium was entirely replaced with a fresh one to keep the sink condition. The drug content in the release medium was determined by high performance liquid chromatography (HPLC, Waters Separation Module e2695) equipped with a C18 column (5 μm, 150 mm × 4.6 mm, Phenomenex) and a Waters UV/Visible detector 2486. The mobile phase was water/acetonitrile (50/50 *V*/*V*) and its flow rate was 1 mL/min with column temperature of 25 °C.

Surgical Procedure

An adult rat laminectomy mode was constructed according to the protocol published elsewhere[55]. Briefly, after general anesthesia was induced, a longitudinal midline skin incision was performed over the L2−L4 levels. Then, the paravertebral muscles were dissected subperiosteally to expose the L2−L4 laminae. Subsequently, all rats underwent a complete L3 laminectomy and the intact dura mater was left. These rats were randomly assigned into 5 groups with 7 rats per group. The 5 groups were set as follows: in the control group (Group A), NS solution was just used to irrigate the epidural space 3 times; in the hydrogel-treated group (Group B), 0.1 mL of the PLGA-PEG-PLGA aqueous solution (25 wt% in NS) was topically applied to the exposed epidural space and around the freed nerve roots; in the other three groups received the treatment of hydrogels containing different DEX amounts (Group C: 1 mg/mL, Group D: 2 mg/mL; Group E: 4 mg/mL), the same volume of DEX-loaded polymer solution (0.1 mL) was administrated. Afterwards, the muscles and the skin were sewed in layers using sterile No. 2-0 silk sutures. The rats were returned to cages and raised carefully during the post-operative period. Finally, they were sacrificed by intraperitoneal injection of pentobarbital (150 mg/kg) at 4 weeks post-operation.

Clinical Observation

For all rats, the process of wound healing was observed, and the neurological functional status at the moment of revival 2 h post-operation and subsequent time points was noted until they were euthanized 4 weeks later. The method described by Liu *et al*.[6] was adopted to evaluate the motion function of the hind limbs. The detailed criteria were described as follows: 1) The posture of the affected hindlimb: 0 = complete deformation, 1 = important deformation; 2 = slight deformation; and 3 = normal; and 2) the force and use of the hindlimb: 0 = complete paralysis; 1 = the hindlimb muscles can be contracted but the limb cannot be mobilized; 2 = hindlimb can be moved horizontally but cannot support the body weight; 3 = hindlimb can support the body weight but with some difficulty; 4 = force and use of the hindlimb are nearly normal; and 5 = force and use of the limb are normal. Animals, whose neural roots were injured in operations, were finally excluded in the following study.

Macroscopic Observation of Epidural Scar Adhesion

One rat in each group was randomly selected and sacrificed 4 weeks after surgery. Then, the qualitatively evaluation of laminectomy site was performed in terms of the inflammation response in the surrounding tissues, the degree of difficulty of re-exposing the dura mater and the amount of epidural fibrosis. The remaining 6 animals in each group were used for histological examination. After the induction of euthanasia, the whole spine columns including the surrounding muscle tissues were resected from L2 to L4, fixed in 4% formalin for one

week, and then decalcified by ethylenediamine tetraacetic acid (EDTA) for 4 weeks. Next, the specimens were cut transversely and only the L3 vertebras were reserved, dehydrated and embedded in paraffin. Two successive transversal sections of each specimen were made in the middle of L3 vertebras, and stained with hematoxylin-eosin (HE) and Masson's trichrome dye, respectively.

All sections were microscopically evaluated by a blinded pathologist. Sections stained with HE were used to examine the extent of adhesion, the density of the fibroblasts and the blood vessels. The extent of adhesion was assessed according to the semi-quantitative classification described by He *et al*. as follows: grade 0, dura mater is free of scar tissue; grade 1, only thin fibrous bands are observed between the scar tissue and dura mater; grade 2, continuous adherence is observed in less than two-thirds of the laminectomy defect; and grade 3, scar tissue adherence is large, affecting more than two-thirds of the laminectomy defect, or the adherence extended to the nerve roots[55]. Three vascularized fields in each section were selected to analyze blood vessel density and each field was 6.25×10^4 μm^2. The number of blood vessels was counted in each field, and then the average vessel density of each section was achieved. Meanwhile, the density of fibroblasts was similarly counted in the corresponding fields. The sections stained with Masson's trichrome were used to evaluate the area of fibrosis between the paraspinal muscles and dura mater, which were recorded using Image-Pro Plus 6.0 software (Media Cybernetics, Inc.). All sections were measured 3 times, and the average area was determined.

All the data were statistically analyzed using SPSS software (version 17.0, SPSS, Inc.). The non-parametric Mann-Whitney *U* test was employed for analysis of the extent of adhesion. And One-Way ANOVA and the LSD test were used to analyze the area of fibrosis, fibroblast density, and blood vessel density. In this study, $p < 0.05$ is considered statistically significant.

RESULTS

Temperature-responsive Gelling Behavior of the PLGA-PEG-PLGA Solutions with or without Drugs in NS

The PLGA-PEG-PLGA triblock copolymer used in this study was synthesized by us and its molecular parameters determined by ^{1}H-NMR and GPC measurements are summarized in Table S1 in Supporting Information. The polymers were easily dissolved in water at low or room temperature, and then the polymeric aqueous solution spontaneously transformed into a semi-solid gel with increasing temperature, if its concentration was greater than the critical gel concentration (CGC). The phase diagram of PLGA-PEG-PLGA with NS as the medium is presented in Fig. S1. We confirm that its CGC was approximately 8 wt% and the sol-gel transition temperature of 25 wt% polymer aqueous solution was about 32 °C.

Rheological measurements were further carried out to assess the temperature-responsive gelling behavior of PLGA-PEG-PLGA solutions with or without drugs in NS. Figure 2(a) shows the storage modulus *G*' and loss modulus *G*" of the polymeric aqueous solutions as a function of temperature. At lower temperatures, both *G*' and *G*" values were quite low, and *G*" was higher than *G*'. This feature demonstrates that the polymeric aqueous system presented a sol state. As the temperature was raised, an abrupt increase in both *G*' and *G*" with several orders of magnitude was observed, and *G*' value exceeded *G*" value at 33 °C, reflecting the *in situ* gel formation. The introduction of different amounts of DEX had no obvious effect on the gelling properties of PLGA-PEG-PLGA triblock copolymers in NS. Figure 2(b) displays the complex viscosity η^* of PLGA-PEG-PLGA solutions with or without drugs in NS as a function of temperature. The η^* changed in a similar way of modulus. In particular, all the η^* values of the polymer systems were less than 0.1 Pa·s at lower temperatures, indicating that they were easily injected through a conventional syringe.

In vitro Hemocompatibility of the PLGA-PEG-PLGA Triblock Copolymer

As an anti-adhesive barrier device after surgery, the PLGA-PEG-PLGA hydrogel needs to cover the injury site and thus its hemocompatibility should be taken into consideration. In this study, the hemolysis test of PLGA-PEG-PLGA polymer at different concentrations was first examined. As shown in Fig. 3, the completed hemolysis, which was defined as 100%, occurred in the group of distilled water, while the hemolysis percentage for all the polymer samples was lower than 5%, meeting the protocol of ISO 10993 as an international standard for the biological evaluation of medical devices.

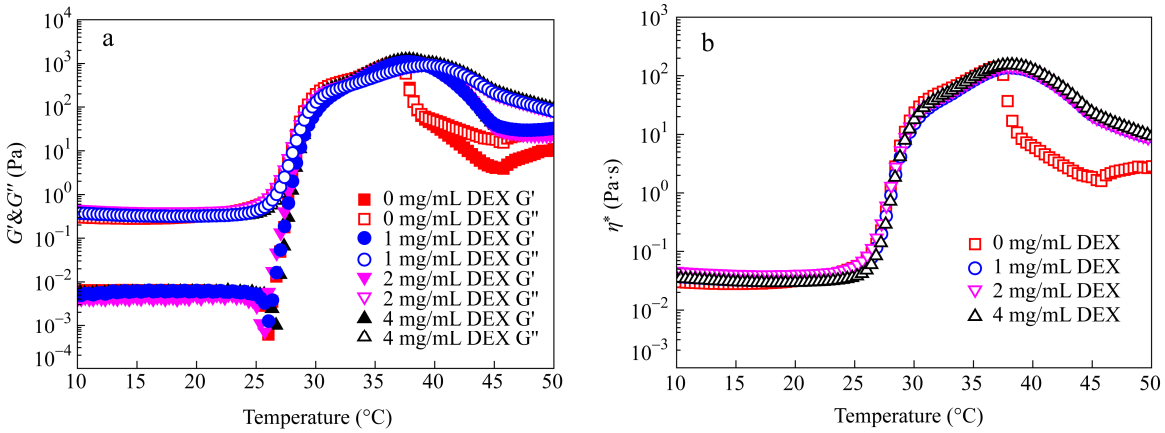


Fig. 2 (a) Storage modulus G' and loss modulus G'' and (b) complex viscosity η^* of the PLGA-PEG-PLGA aqueous solutions (25 wt% in NS) with or without drugs as a function of temperature (Heating rate: 0.5 K/min; oscillation frequency: 10 rad/s)

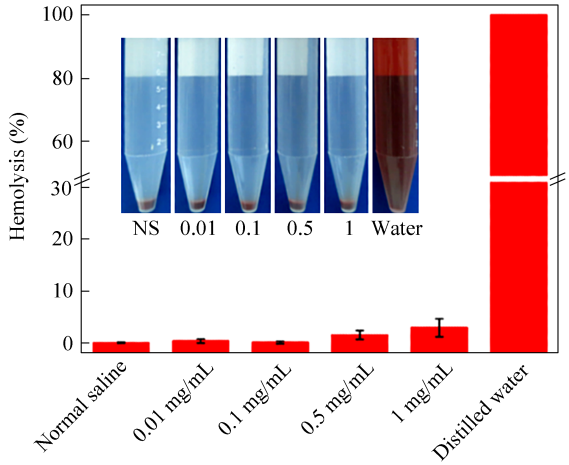


Fig. 3 Hemolysis test of PLGA-PEG-PLGA aqueous solutions at indicated concentrations
The distilled water was set as the positive group, while the NS solution was set as the negative group. The inserted image shows the medium color of hemolysis of red blood cells after centrifugation (n = 3).

In vitro Degradation of the PLGA-PEG-PLGA Hydrogel

As an implanted biomaterial, its biodegradability is important and should be concerned. *In vitro* degradation of the thermo-reversible PLGA-PEG-PLGA hydrogel was performed under physiological conditions (pH 7.4, 37 °C). Figure 4(a) presents the volume change of remaining hydrogels at the indicated time points. The polymer hydrogel swelled continuously and its volume increased over 100% after incubation in PBS at 37 °C for 5 days. Afterwards, the swelling rate of hydrogel slightly slowed down. Figure 4(b) presents the weight change of remaining hydrogels as a function of degradation time. The weight of hydrogel decreased to about 50% after 5 weeks of degradation. The GPC traces of the PLGA-PEG-PLGA hydrogel during *in vitro* degradation period are displayed in Fig. 4(c). A slow increase in retention time at peak maximum and the peak appearance of degradation products of low MWs demonstrated a gradual MW decrease of residual polymers, which was mainly caused by the hydrolysis of ester bonds in the PLGA chains[53]. Also, the GPC traces gradually broadened, indicating the increase in the MW distributions of residual polymers during the examined degradation period. Figure 4(d) quantitatively shows the decreased peak MW (M_{peak}) and increased MW distribution following *in vitro* degradation. In fact, besides the hydrolysis of PLGA chains, the surface erosion is another significant pathway of *in vitro* degradation of the PLGA-PEG-PLGA hydrogels in PBS[53, 56]. Meanwhile, the buffer

solution was replaced by a fresh one per 5 days in the present study. Consequently, the PLGA-PEG-PLGA copolymers and their degradation compounds of low MWs continuously entered the buffer solution due to the hydrolysis of PLGA chains and the surface erosion of the hydrogel, resulting in the half mass loss of polymer after 5 weeks of degradation. Changes in the pH of the buffer medium were also detected throughout the degradation process, as shown in Fig. 4(e). The pH value of the medium decreased from 7.4 to 6.9 within the initial 5 days. Subsequently, the medium pH only fluctuated within a narrow range.

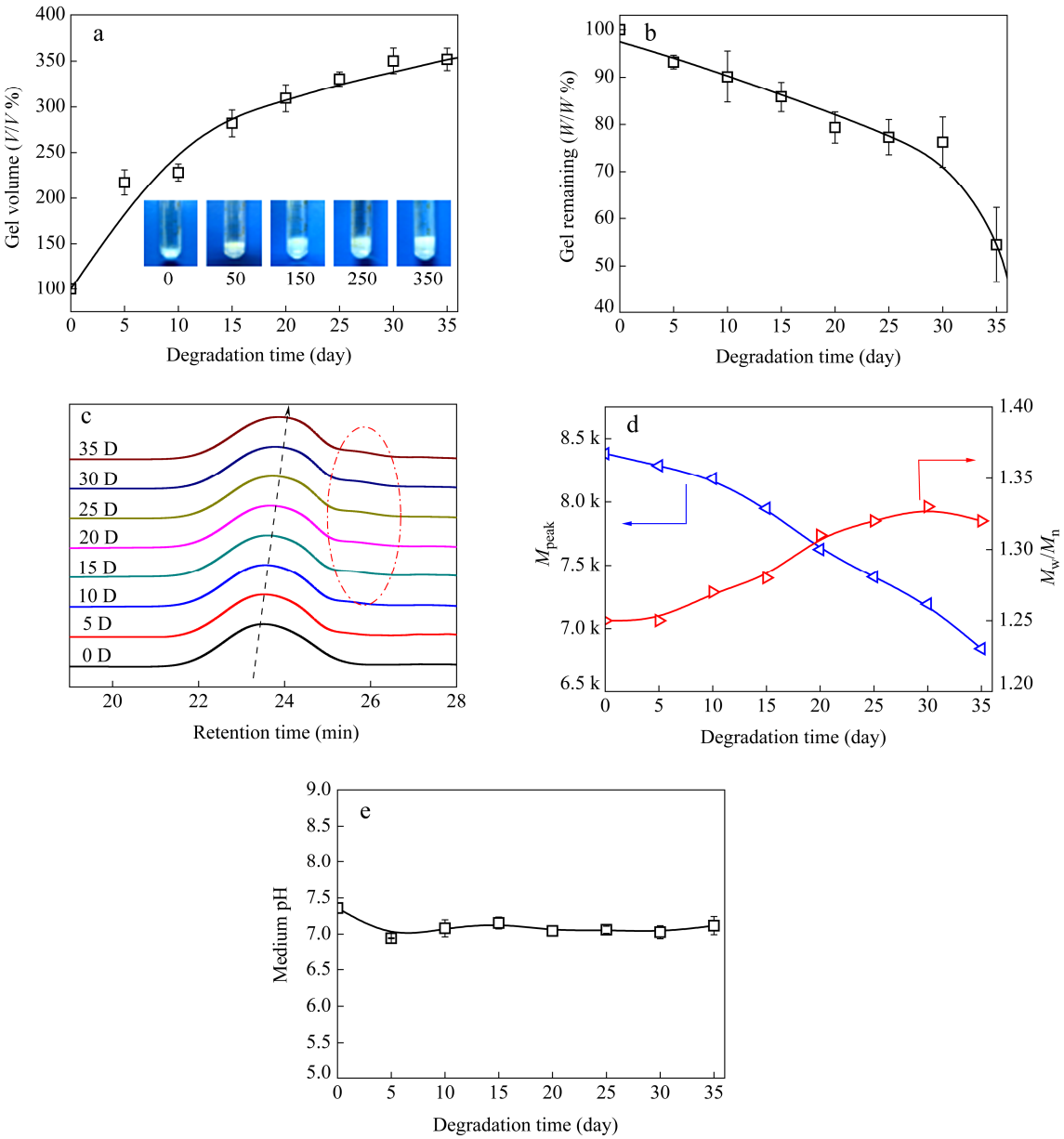


Fig. 4 *In vitro* degradation of the PLGA-PEG-PLGA hydrogel in PBS (pH 7.4) at 37 °C: (a) Time-dependent remaining volume of the polymer hydrogels with inserted photographs presenting the global observation of the hydrogels before and after degradation; (b) Time-dependent remaining mass of the polymer hydrogels; (c) GPC traces before and after the indicated degradation period *in vitro* with dashed arrow representing the decrease of M_{peak} and dashed circle emphasizing the peaks of degradation products of low MWs; (d) Time-dependent M_{peak} and MW distribution (M_w/M_n) at day 0 and in degradation, respectively (The data were detected from GPC); (e) Time-dependent pH values of the buffer medium

***In vivo* Degradation of the PLGA-PEG-PLGA Hydrogel**

In vivo degradation of the injectable PLGA-PEG-PLGA hydrogel was examined in SD rats. After subcutaneous injection of the copolymer aqueous solution using a 1 mL syringe with a 25-gauge needle, the polymer/water system spontaneously formed an *in situ* hydrogel at the administration site, as shown in Fig. 5(a). Then, the remaining gels under the skins were dissected and taken out at the predetermined intervals. Figure 5(b) shows the change in size of remaining gels as a function of degradation time. Due to the formation of a fibrous capsule enclosing the hydrogel and some muscle adhering to the fibrous capsule after dissection, the size of hydrogel after 7 days was larger than that obtained at 30 min post-injection. Afterwards, the size of gel gradually decreased during the degradation period. The integrity of the PLGA-PEG-PLGA hydrogel maintained over one month. The remaining gels obtained were further used for mass measurement and GPC detection. The trend of mass change of remaining gels as a function of degradation time was consistent with the change of size, as displayed in Fig. 5(c). The GPC profiles with degradation time showed that the intensities of the initial PLGA-PEG-PLGA peak weakened during the degradation period, as presented in Fig. 5(d). However, several low MW peaks, which were assigned to degradation species, appeared following the degradation and their intensities increased over time. Meanwhile, no sign of tissue necrosis, hemorrhaging, or hyperemia dema was observed at the injection site during the whole period of examination.

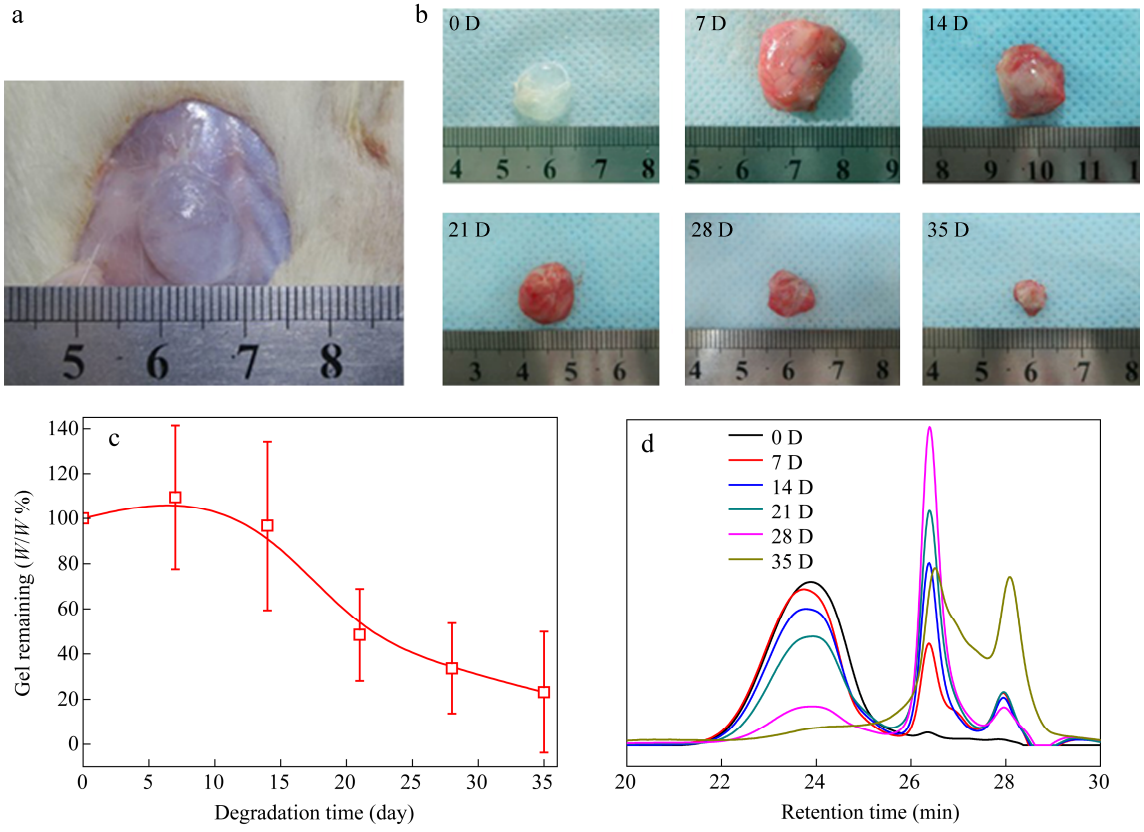


Fig. 5 *In vivo* degradation of the PLGA-PEG-PLGA hydrogel subcutaneously implanted in SD rats: (a) The *in situ* formation of hydrogel under the skin at 30 min post-injection; (b) Macroscopic observation of the gel morphology as a function of degradation time; (c) Remaining mass of the polymer hydrogels during degradation; (d) GPC traces of the remaining gels during degradation

***In vitro* Drug Release from the PLGA-PEG-PLGA Hydrogel**

The *in vitro* release profiles of the PLGA-PEG-PLGA hydrogels containing DEX were examined under physiological conditions (pH 7.4, 37 °C), as shown in Fig. 6. The drug exhibited a sustained release manner up

to 10 days. Both the initial burst and the cumulative released amount were dependent on the DEX loading content. The high drug loading content resulted in a small burst and a low cumulative released rate.

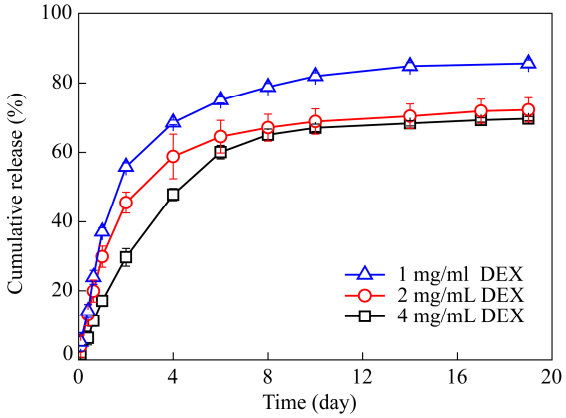


Fig. 6 *In vitro* release of DEX from the PLGA-PEG-PLGA hydrogel
The polymer concentration was 25 wt%. Error bars represent the standard deviation (n = 3).

Macroscopic Observation of Specimens in Animal Experiments

In the present study, a total L3 laminectomy was performed on thirty-five SD rats. All incisions healed within the initial one week and no infections were observed throughout the experiment period. All the animals were healthy and ambulatory without evidence of neurological deficit after surgery. Meanwhile, the motion function of the affected hind limbs of rats after surgery was evaluated. The results demonstrate that all the hind limbs exhibited normal function with the posture of Grade 3 (normal posture) and the force of Grade 5 (normal force and use of the limb).

After the induction of euthanasia at 4 weeks post-operation, the necropsies of rats revealed that no animal suffered from serious inflammation, abscess or necrosis at the laminectomy site or the surrounding soft tissues.

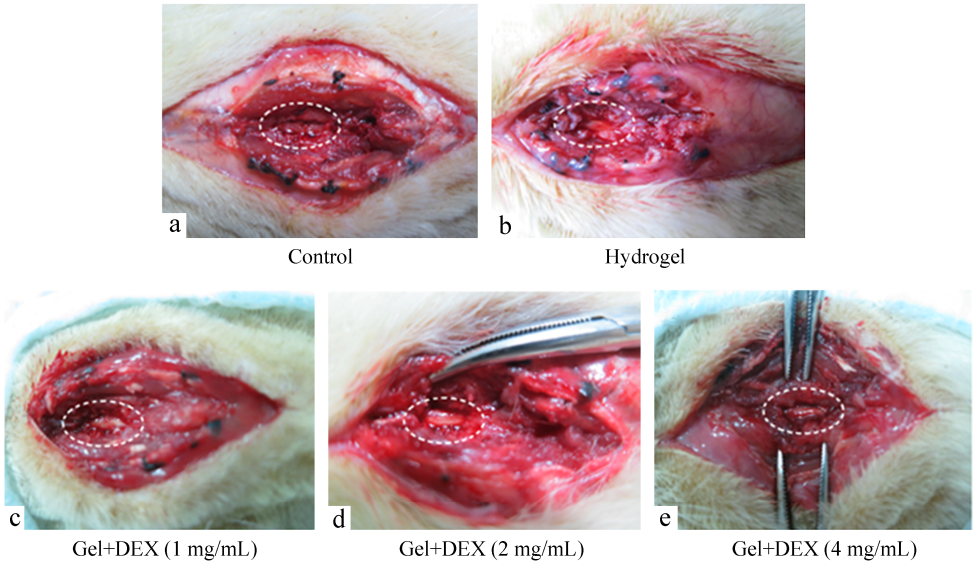


Fig. 7 Macroscopic observation of the laminectomy site: (a–e) gross anatomical observations of the epidural scar tissues at one month post-laminectomy
(b and d) a small amount of loose epidural scar tissues formed at the laminectomy site and could be easily removed to re-expose the dura mater. (a, c and e) tenacious epidural scar tissues directly adhered to the dural sac, resulting in the difficulty of re-exposing the dura mater.

This finding was attributed to the good biocompatibility of PLGA-PEG-PLGA hydrogel. Meanwhile, in both the groups received the treatment of hydrogel (Group B) or DEX-loaded hydrogel (Group D: 2 mg/mL), a small amount of loose epidural scar tissues was observed, and it was easy to re-expose the dural sac clearly and intactly. However, the thick and tenacious epidural scar adhesions were detected in the control group (Group A) and the other two DEX-loaded hydrogel-treated groups (Groups C: 1 mg/mL and E: 4 mg/mL), and it was difficult to remove the scar tissues from the dura mater, as shown in Fig. 7.

General Findings of Histologic Sections

We spent several months to obtain the HE-stained and Masson-stained histologic sections, and some representative images are shown in Fig. 8. In both the groups B and D, loose collagen fibrils filled the

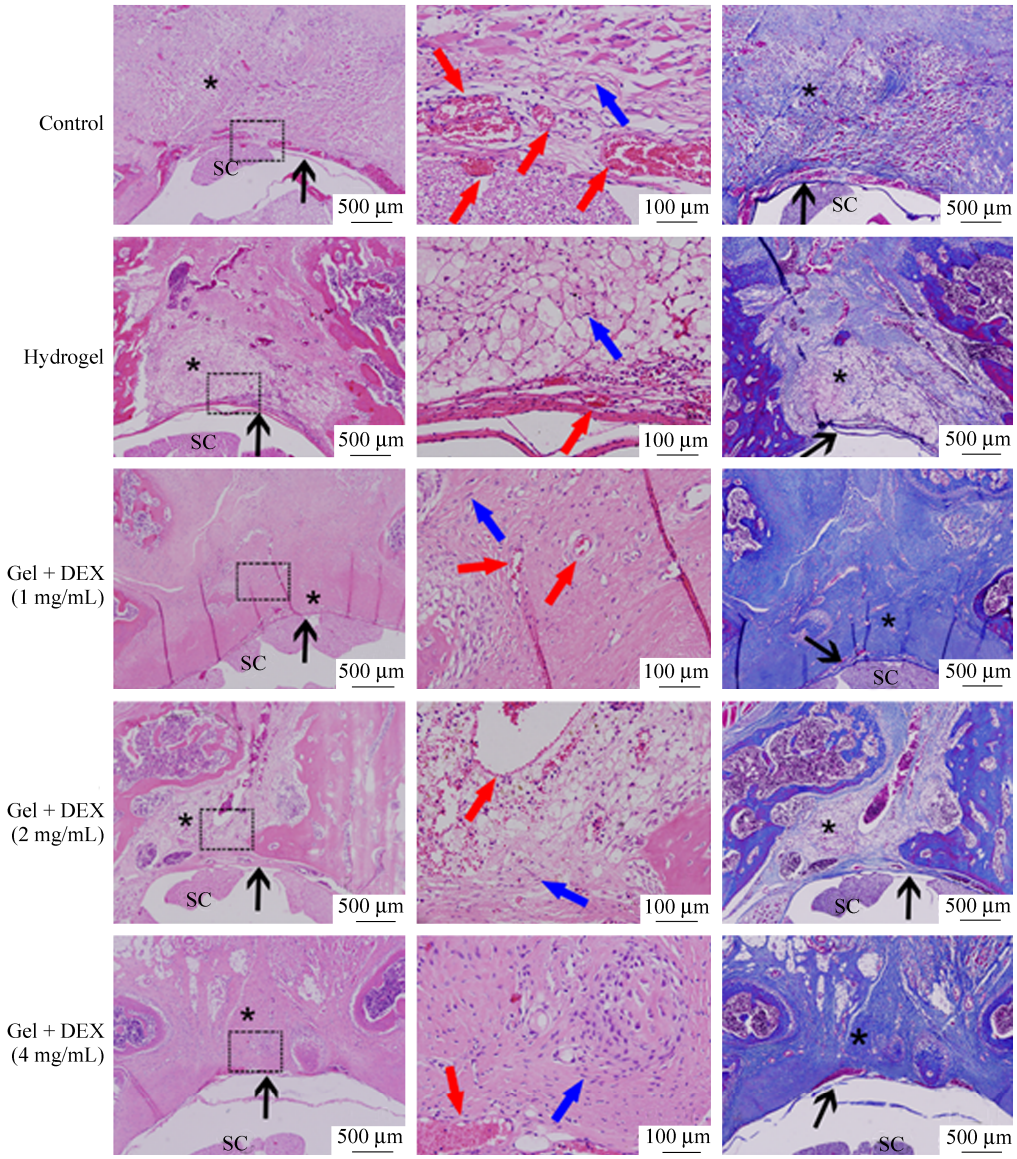


Fig. 8 Representative histologic sections with HE staining (left) and corresponding histologic sections with Masson staining (right) in the middle of laminectomy sites after 4 weeks of surgery
Black arrows represent dura mater, and asterisks represent scar tissue. SC: spinal cord. The dashed rectangles in the left images indicate the regions presented in the middle images with a higher magnification. Blue arrow: fibroblast; Red arrow: blood vessel.

laminectomy defects in strips without tenacious scar tissues attaching to the dura mater directly. In contrast, in the other three groups (Groups A, C and E), a mass of dense, thick epidural scar tissues deposited at the surgical sites and adhered to the dura mater directly and closely. Meanwhile, the histological evaluation also demonstrates that there was no obvious inflammatory cell infiltration at the laminectomy site with the administration of various hydrogel systems.

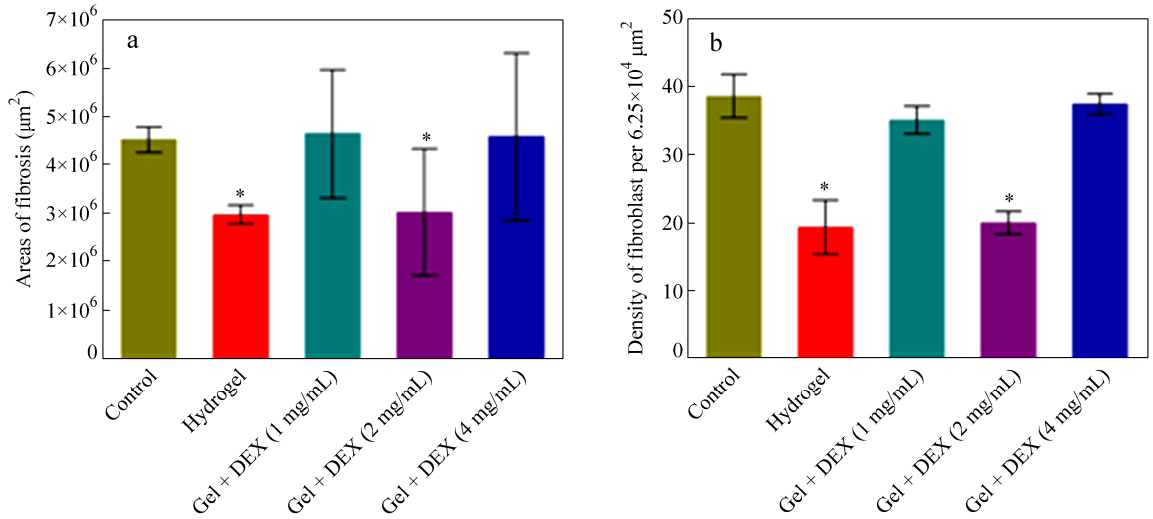
Histological Evaluation

The extent of epidural fibrosis for all the 5 groups was graded, and the results are listed in Table 1. The extent of epidural fibrosis in the group B and the group D was remarkably inferior to that in the other three groups. There were statistically significant differences in terms of the extent of epidural fibrosis for the group B compared with the group A, group C and group E ($p < 0.05$). Also, significant differences exited for the group D compared with the group A, group C and group E ($p < 0.05$). Nevertheless, there was no statistically significant difference among the group A, group C and group E. Meanwhile, no statistically significant difference was observed between the group B and the group D.

Table 1. Grading for the extent of epidural fibrosis

	Grade 0	Grade 1	Grade 2	Grade 3
Group A: Control	0	0	0	6
Group B: Hydrogel	0	4	2	0
Group C: Gel + DEX (1 mg/mL)	0	0	1	5
Group D: Gel + DEX (2 mg/mL)	0	3	3	0
Group E: Gel + DEX (4 mg/mL)	0	1	0	5

Finally, the quantitative analysis of the area of epidural fibrosis, number of fibroblasts, and blood vessel density for all the 5 groups was also carried out with the results displayed in Fig. 9. Both the areas of epidural fibrosis and density of fibroblasts in the group B were significantly reduced compared with those in the group A, group C and group E after 4 weeks of surgery ($p < 0.05$). The reduction trend was also seen in the group D compared with those in the group A, group C and group E ($p < 0.05$). The density of the blood vessel only in the control group (Group A) was obviously higher than those in the other four groups received the treatment of hydrogels with or without drugs ($p < 0.05$).



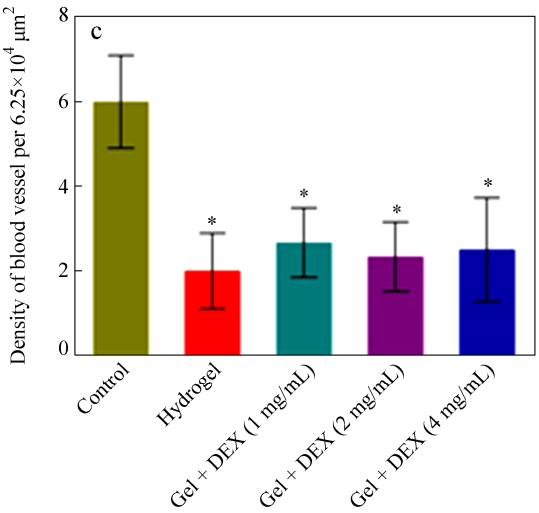


Fig. 9 Area of fibrosis (a), density of fibroblasts (b) and blood vessel density (c) in the five groups after 4 weeks of surgery Significant differences between the control group and the other four groups are specifically marked: "*", $p < 0.05$

DISCUSSION

The formation of epidural scar tissues is almost inevitable after spine surgeries. Based on preclinical and clinical researches, post-operative peridural fibrosis or adhesion is the causative factor in most cases of recurrent radicular or back pain due to compression and stretching of the exposed dura and associated nerve roots, which drastically devastates the compliance of patients undergoing spine surgery[3, 57]. What's more, epidural scarring or fibrosis increases the technical difficulty and risk of subsequent surgeries. In some cases, epidural fibrosis was even more severe after repeated operations[58]. Prevention of epidural fibrosis has been a major challenge in spine surgery.

Although the pathological mechanism of epidural fibrosis is very complex, it is commonly acknowledged that the trauma and the formation of epidural hematoma stimulate the migration of fibroblasts from the paravertebral muscles into the laminectomy defect, resulting in secreting largely extracellular matrix components, such as collagen and fibronectin, and finally the hematoma is replaced with tenacious scar tissue[3, 6]. One strategy of prevention of epidural fibrosis is to interpose a kind of anti-adhesion materials between the dura mater and the paravertebral muscle to mechanically isolate the dura mater from the gradually produced scar tissue. To date, both natural matrices, such as free fat transplantation[59], carbohydrate polymer gels composed of polyglycan ester and porcine-derived gelatin (ADCON-L, Gliatech, USA)[5], collagen-based sealant (Imedex, Chaponost, France)[6], hyaluronic acid-carboxymethyl cellulose film (Seprafilm, Genzyme, USA)[60], and synthetic materials such as polylactide film[61] and polytetrafluoroethylene membrane[62] have been utilized in experimental studies. However, the most of materials only achieved limited success.

Considering that the trauma of peridural tissues in spine surgery may be a vital factor of scar formation[63], the second strategy is based on the use of anti-inflammation agents or anti-proliferation drugs to inhibit the inflammation or proliferation of fibroblasts and thus prevent the formation of epidural adhesion. In the past decades, various drugs have been evaluated experimentally but controversial results with positive and negative findings were obtained[8, 10, 64, 65]. Glucocorticoids, due to their good anti-inflammatory and immune-modulating effects, were universally used in medical practice[66–68]. In spine surgery, they were usually used perioperatively to improve treatment outcomes[67]. For example, Debi *et al*. demonstrated that the local application of methylprednisolone acetate following lumbar discectomy effectively decreased the post-operative back pain of patients[68]. The same drug was also tried to prevent epidural fibrosis in a rat model, however, only a negative efficacy was observed[65].

Thermo-sensitive polymer hydrogels are unique barrier materials due to the ability to conveniently cover the whole dura mater before gelation and then spontaneously form an *in situ* hydrogel at the defect site to play a physical barrier after laminectomy. The efficacy of some thermo-reversible hydrogels of PEG and biodegradable polyesters in preventing post-surgical peritoneal adhesions has been demonstrated and the possible mechanisms were also proposed by us[40, 46].

In this study, an injectable and biodegradable PLGA-PEG-PLGA hydrogel was prepared and used as both the barrier device and the drug delivery carrier to prevent the peridural scar formation after laminectomy. The concentrated polymer aqueous solution underwent a sol-gel transition as the temperature increased and the gel window covered physiological temperature, as illustrated in Fig. S1 and Fig. 2. Its basic mechanism of sol-gel transition was attributed to the formation of a percolated micelle network[28, 35].

The *in vitro* hemolysis assay affirms that the synthesized polymer has low hematotoxicity (Fig. 4). The *in vitro* and *in vivo* degradation experiments confirm that the resulting PLGA-PEG-PLGA hydrogel could maintain its integrity as long as several weeks (Fig. 4 and Fig. 5). The final degradation products are lactic acid, glycolic acid and PEG[53, 56]. We also notice that the degradation period of hydrogel *in vivo* was faster than that *in vitro* (Fig. 4 and Fig. 5). The effect of enzyme and other factors *in vivo* accelerate the degradation of hydrogel[56].

The thermo-reversible PLGA-PEG-PLGA hydrogels have been tried to delivery various therapeutic agents, such as hydrophobic drugs, hydrophilic drugs and proteins[36, 44, 69]. For example, the PLGA-PEG-PLGA hydrogel formulation containing paclitaxel, a hydrophobic anticancer drug, exhibited a sustained release manner over 50 days and the gel degradation governed the drug release[69]. Also, lysozyme as a modern protein was incorporated into the PLGA-PEG-PLGA hydrogel and the dynamics of drug release was relied on the degradation process as well[56]. However, due to its good water-solubility, cisplatin easily diffused into the release medium, resulting in the complete release of drug from the gel matrix within 12 h[22]. These findings indicate that the PLGA-PEG-PLGA hydrogel is more suitable as the delivery carrier of hydrophobic drugs and proteins than hydrophilic agents. In this study, the release of DEX from the PLGA-PEG-PLGA hydrogel lasted up to 10 days, and the initial burst and the cumulative released rate could be modulated *via* the drug loading content, as shown in Fig. 6. Considering a certain solubility of DEX in water (0.1 mg/mL)[70], we believe that the DEX release was governed by the combination of drug diffusion and hydrogel degradation.

A rat lumbar laminectomy model was employed to assess the efficacy of PLGA-PEG-PLGA hydrogel itself and DEX-loaded hydrogel systems in preventing the epidural fibrosis. It is convenient to use the thermo-reversible PLGA-PEG-PLGA hydrogel during spinal surgery. Its polymeric aqueous solution with or without drugs can easily fill the entire laminectomy defect and then spontaneously form a semi-solid gel at the administration site. Because the initial 4 weeks after surgery played an important role in epidural scar formation[71], gross anatomical observations and histological examinations were performed at one month post-laminectomy. Post-operation observation confirms that the liberty of nerve roots was not affected by the treatment of hydrogels with or without drugs, indicating that the hydrogel system is sufficiently soft and its biocompatibility is very good. Meanwhile, no infection was detected in any groups during the postoperatively examined period.

Both the gross anatomical observations and quantitative histological analysis (Figs. 7–9 and Table 1) demonstrate that the administration of the PLGA-PEG-PLGA hydrogel (Group B) significantly reduced the extent of epidural scar and thus prevented scar tissues adhering to the dura mater and the surrounding nerve roots. In contrast, the dense, thick epidural adhesions were observed in the control group (Group A).

Theoretically, the DEX-loaded hydrogel systems could be more effective than the hydrogel alone (Group B) due to the combination of drug treatment and material barrier. However, except the group received the treatment of hydrogel system containing 2 mg/mL DEX (Group D), the efficacy of preventing epidural fibrosis was questionable in the other two DEX-loaded hydrogel groups (Group C with 1 mg/mL DEX and Group E with 4 mg/mL DEX) and there was no statistically significant difference between the two groups and the control group. This feature reveals the irregular dose-dependency of DEX in preventing epidural adhesion after

laminectomy. In fact, such an irregular dose-dependency of DEX in the treatment of other illness have been reported. For example, Czarnetzki *et al*. found that the increased dose of DEX resulted in the reduced risk of post-operative nausea and vomiting, but caused an increased risk of post-operative hemorrhage for children undergoing tonsillectomy[72]. The present study also indicates that the mechanism of glucocorticoids in preventing epidural fibrosis is fairly complex. In the future work, the *in vivo* efficacy of DEX alone on epidural fibrosis prevention and the corresponding hydrogel formulations with the wider dose range of drug need to be investigated. These study will be helpful to further make clear the irregular drug dose-dependent *in vivo* efficacy.

CONCLUSIONS

A thermo-sensitive and biodegradable PLGA-PEG-PLGA hydrogel and the corresponding DEX-loaded hydrogel formulations were tried as a mechanical barrier device or a composited system of drug and material for prevention of epidural scar formation in rats. The concentrated polymeric aqueous solution was a free-flowing liquid at ambient temperature, and an *in situ* hydrogel was rapidly formed at the administration site of warm-blooded animal after injection. This polymer just presented a very low hemolysis. Meanwhile, both the *in vitro* and *in vivo* tests demonstrate that the integrity of polymer hydrogel could be maintained over one month. The introduction of DEX had no effect on the sol-gel transition of PLGA-PEG-PLGA aqueous solution and a sustained release of drug from the hydrogel matrix was achieved up to 10 days.

In vivo experiments confirm that the hydrogel alone effectively reduced epidural scar formation, indicating that the PLGA-PEG-PLGA hydrogel is suitable as a physical barrier device in spine surgery. Nevertheless, the sustained delivery of DEX in the laminectomy defect after surgery exhibited an irregular dose-dependency in preventing epidural fibrosis. Post-surgical epidural scarring could be inhibited only with an appropriate dose of drug. These findings remind the researchers to be careful when summarizing a drug efficacy just based upon one or two drug doses. Meanwhile, the irregular drug dose-dependent *in vivo* efficacy reported by us may be helpful for other researchers to design their experiments.

REFERENCES

1 Malmivaara, A., Slatis, P., Heliovaara, M., Sainio, P., Kinnunen, H., Kankare, J., Dalin-Hirvonen, N., Seitsalo, S., Herno, A., Kortekangas, P., Niinimaki, T., Ronty, H., Tallroth, K., Turunen, V., Knekt, P., Harkanen, T., Hurri, H. and Finnish Lumbar Spinal Res. Grp., Spine, 2007, 32: 1

2 Samy, A.M. and Hardy, R.J., Neurol. Res., 1999, 21 Suppl 1: S5

3 Abitbol, J.J., Lincoln, T.L., Lind, B.I., Amiel, D., Akeson, W.H. and Garfin, S.R., Spine, 1994, 19: 1809

4 Rowlingson, J., Anesth. Analg., 1999, 88: 240

5 Einhaus, S.L., Robertson, J.T., Dohan, F.C., Wujek, J.R. and Ahmad, S., Spine, 1997, 22: 1440

6 Liu, S., Boutrand, J.P. and Tadie, M., J. Neurosurg., 2001, 94: 61

7 Topsakal, C., Akpolat, N., Erol, F.S., Ozveren, M.F., Akdemir, I., Kaplan, M., Tiftikci, M. and Kilic, N., J. Neurosurg., 2004, 101: 295

8 Yan, L., Li, X., Wang, J., Sun, Y., Wang, D., Gu, J., He, J., Hu, H., Chen, G., Wang, Q. and Feng, X., Eur. J. Pharmacol., 2013, 699: 194

9 Karatay, M., Erdem, Y., Koktekir, E., Erkoc, Y.S., Caydere, M. and Bayar, M.A., Turk. Neurosurg., 2012, 22: 753

10 Sandoval, M.A. and Hernandez-Vaquero, D., Eur. Spine J., 2008, 17: 451

11 Mastronardi, L., Pappagallo, M., Puzzilli, F. and Tatta, C., Neurosurgery, 2002, 50: 518

12 Kim, K.D., Wang, J.C., Robertson, D.P., Brodke, D.S., Olson, E.M., Duberg, A.C., BenDebba, M., Block, K.M. and diZerega, G.S., Spine, 2003, 28: 1080

13 Rhyne, A.L., Blumenthal, S.L., Frank, E.H., Hsu, K.Y., Kim, K.D., Youssef, J.A., Wang, J.C., Arnold, P., BenDebba, M., Block, K.M., Juarez, T.G., Chiacchierini, R.P., Ehmsen, R.J., Krelle, J.S., diZerega, G.S. and Oxiplex Clinical Study

Grp., Spine, 2012, 37: 631
14 Fan, C.J., Liao, L.Q., Zhang, C. and Liu, L.J., J. Mater. Chem. B., 2013, 1: 4251
15 Wu, L.Y., Yu, L., Fu, X.H. and Li, Z.B., Chinese J. Polym. Sci., 2015, 33(8): 1140
16 Zhao, Z.B., An, S.S., Xie, H.J. and Jiang, Y., Chinese J. Polym. Sci., 2015, 33(1): 173
17 Zeng, W., Hu, W.K., Li, H., Jing, Y.H., Kang, H., Jiang, Q. and Zhang, C., Chinese J. Polym. Sci., 2014, 32(11): 1507
18 Cigognini, D., Silva, D., Paloppi, S., and Gelain, F., J. Biomed. Nanotechnol., 2014, 10: 309
19 Moon, H.J., Ko, D.Y., Park, M.H., Joo, M.K. and Jeong, B., Chem. Soc. Rev., 2012, 41: 4860
20 Yu, L. and Ding, J.D., Chem. Soc. Rev., 2008, 37: 1473
21 Zhang, Y.B., Ding, J.X., Xu, W.G., Wu, J., Chang, F., Zhuang, X.L., Chen, X.S. and Wang, J.C., Chinese J. Polym. Sci., 2014, 32(12): 1590
22 Shen, W.J., Luan, J.B., Cao, L.P., Sun, J., Yu, L. and Ding, J.D., Biomacromolecules, 2015, 16: 105
23 Zhang, Y.B., Ding, J.X., Sun, D.K., Sun, H., Zhuang, X.L., Chang, F., Wang, J.C. and Chen, X.S., Mater. Sci. Eng. C-Mater. Biol. Appl., 2015, 49: 262
24 Ni, P., Ding, Q.X., Fan, M., Liao, J.F., Qian, Z.Y., Luo, J.C., Li, X.Q., Luo, F., Yang, Z.M. and Wei, Y.Q., Biomaterials, 2014, 35: 236
25 Li, Z.B., Zhang, Z.X., Liu, K.L., Ni, X.P. and Li, J., Biomacromolecules, 2012, 13: 3977
26 Liu, C.D., Zhang, Z., Liu, K.L., Ni, X. and Li, J., Soft Matter, 2013, 9: 787
27 Shim, M.S., Lee, H.T., Shim, W.S., Park, I., Lee, H., Chang, T., Kim, S.W. and Lee, D.S., J. Biomed. Mater. Res., 2002, 61: 188
28 Yu, L., Chang, G.T., Zhang, H. and Ding, J.D., J Polym. Sci., Part A: Polym. Chem., 2007, 45: 1122
29 Chen, C., Chen, L., Cao, L.P., Shen, W.J., Yu, L. and Ding, J.D., Rsc. Adv., 2014, 4: 8789
30 Kwon, J.S., Kim, S.W., Kwon, D.Y., Park, S.H., Son, A.R., Kim, J.H. and Kim, M.S., Biomaterials, 2014, 35: 5337
31 Park, M.H., Choi, B.G. and Jeong, B., Adv. Funct. Mater., 2012, 22: 5118
32 Park, M.H., Yu, Y., Moon, H.J., Ko, D.Y., Kim, H.S., Lee, H., Ryu, K.H. and Jeong, B., Adv. Healthc. Mater., 2014, 3: 1782
33 Il Kim, J., Kim, B., Chun, C., Lee, S.H. and Song, S.C., Biomaterials, 2012, 33: 4836
34 Cho, J.K., Hong, J.M., Han, T., Yang, H.K. and Song, S.C., J. Drug Target., 2013, 21: 564
35 Yu, L., Zhang, Z., Zhang, H. and Ding, J.D., Biomacromolecules, 2009, 10: 1547
36 Ci, T.Y., Chen, L., Yu, L. and Ding, J.D., Sci. Rep., 2014, 4: 5473
37 Chen, L., Ci, T.Y., Li, T., Yu, L. and Ding, J.D., Macromolecules, 2014, 47: 5895
38 Bae, S.J., Joo, M.K., Jeong, Y., Kim, S.W., Lee, W.K., Sohn, Y.S. and Jeong, B., Macromolecules, 2006, 39: 4873
39 Petit, A., Müller, B., Meijboom, R., Bruin, P., van de Manakker, F., Versluijs-Helder, M., de Leede, L.G.J., Doornbos, A., Landin, M., Hennink, W.E. and Vermonden, T., Biomacromolecules, 2013, 14: 3172
40 Zhang, Z., Ni, J., Chen, L., Yu, L., Xu, J.W. and Ding, J.D., Biomaterials, 2011, 32: 4725
41 Yu, L., Zhang, H. and Ding, J.D., Angew. Chem.-Int. Edit., 2006, 45: 2232
42 Yu, L., Zhang, Z. and Ding, J.D., Biomacromolecules, 2011, 12: 1290
43 Lei, K.W., Shen, W.J., Cao, L.P., Yu, L. and Ding, J.D., Chem. Commun., 2015, 51: 6080
44 Chang, G.T., Ci, T.Y., Yu, L. and Ding, J.D., J., Control. Release, 2011, 156: 21
45 Li, K., Yu, L., Liu, X.J., Chen, C., Chen, Q.H. and Ding, J.D., Biomaterials, 2013, 34: 2834
46 Yu, L., Hu, H.T., Chen, L., Bao, X.G., Li, Y.Z., Chen, L., Xu, G.H., Ye, X.J. and Ding, J.D., Biomater. Sci., 2014, 2: 1100
47 Yu, L., Xu, W., Shen, W.J., Cao, L.P., Liu, Y., Li, Z.S. and Ding, J.D., Acta Biomater., 2014, 10: 1251
48 Li, X.Q., Chen, L., Lin, H., Cao, L.P., Cheng, J.A., Dong, J., Yu, L. and Ding, J.D., J. Spinal Disord. Technol., 2015, DOI: 10.1097/bsd.0000000000000221
49 Yang, B., Gong, C.Y., Qian, Z.Y., Zhao, X., Li, Z.Y., Qi, X.R., Zhou, S.T., Zhong, Q.A., Luo, F. and Wei, Y.Q., BMC Biotechnol., 2010, 10: 65
50 Gong, C.Y., Wu, Q.J., Liao, J.F., Qi, T.T., Yang, B., Wang, Y.J., Guo, G., Luo, F., Zhao, X., Wei, Y.Q. and Qian, Z.Y., J. Biomed. Nanotechnol., 2013, 9: 1984

51 Fu, S.Z., Li, Z, Fan, J.M., Meng, X.H., Shi, K., Qu, Y, Yang, L.L., Wu, J.B., Fan, C.J., Luo, F. and Qian, Z.Y., J. Biomed. Nanotechnol., 2014, 9: 427
52 Rhen, T. and Cidlowski, J.A., N. Engl. J. Med., 2005, 353: 1711
53 Yu, L., Zhang, Z. and Ding, J.D., Macromol. Res., 2012, 20: 234
54 Cao, L. P., Cao, B., Lu, C.J., Wang, G.W., Yu, L. and Ding, J.D., J. Mater. Chem. B., 2015, 3: 1268
55 He, Y., Revel, M. and Loty, B., Spine, 1995, 20: 557
56 Yu, L., Zhang, Z., Zhang, H. and Ding, J.D., Biomacromolecules, 2010, 11: 2169
57 Benoist, M., Ficat, C., Baraf, P. and Cauchoix, J., Spine, 1980, 5: 432
58 Fandino, J., Botana, C., Viladrich, A. and Gomezbueno, J., Acta Neurochir., 1993, 122: 102
59 Kiviluoto, O., Acta Orthop. Scand., 1976, 164: 3
60 Topsakal, C., Akpolat, N., Erol, F.S., Ozveren, M.F., Akdemir, I., Kaplan, M., Tiftikci, M. and Kilic, N., J. Neurosurg., 2004, 101: 295
61 Klopp, L.S., Welch, W.C., Tai, J.W., Toth, J.M., Cornwall, G.B. and Turner, A.S., Neurosurg. Focus, 2004, 16: E2
62 Ivanic, G.M., Pink, P.T., Schneider, F., Stuecker, M., Homann, N.C. and Preidler, K.W., Eur. Spine J., 2006, 15: 1360
63 Broughton, G., Janis, J.E. and Attinger, C.E., Plast. Reconstr. Surg., 2006, 117: 12S
64 Ozdemir, O., Calisaneller, T., Sonmez, E., Kiyici, H., Caner, H. and Altinors, N., Neurol. Res., 2010, 32: 1117
65 Cekinmez, M., Sen, O., Atalay, B., Erdogan, B., Bavbek, M., Caner, H., Ozen, O. and Altinors, N., Neurol. Res., 2010, 32: 700
66 Lee, H.W., Lee, H., Chung, H., Park, J.C., Shin, S.K., Lee, S.K., Lee, Y.C., Hong, J.H. and Kim, D.W., Surg. Endosc., 2014, 28: 2334
67 Foulkes, G.D. and Robinson, J.J., Clin. Orthop. Rel. Res., 1990, 224
68 Debi, R., Halperin, N. and Mirovsky, Y., J. Spinal Disord. Tech., 2002, 15: 273
69 Zentner, G.M., Rathi, R., Shih, C., McRea, J.C., Seo, M.H., Oh, H., Rhee, B.G., Mestecky, J., Moldoveanu, Z., Morgan, M. and Weitman, S., J. Control. Release, 2001, 72: 203
70 Dilova, V., Zlatarova, V., Spirova, N., Filcheva, K., Pavlova, A. and Grigorova, P., Boll. Chim. Farm., 2004, 143: 20
71 Cekinmez, M., Erdogan, B., Tufan, K., Sarica, F.B., Ozen, O. and Caner, H., Neurol. Res., 2009, 31: 322
72 Czarnetzki, C., Elia, N., Lysakowski, C., Dumont, L., Landis, B.N., Giger, R., Dulguerov, P., Desmeules, J. and Tramer, M.R., Jama-J. Am. Med. Assoc., 2008, 300: 2621