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RESEARCH ARTICLE

5-Fluorouracil delivery from a novel three-dimensional micro-device: in vitro and in vivo evaluation

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Abstract A novel three-dimensional biodegradable micro-device using microelectromechanical systems technology was developed for implantable controlled drug delivery. In order to evaluate the effect of monomer composition and molecular weight of poly(lactic-*co*-glycolic acid) (PLGA) on the drug release, three 5-Fluorouracil loaded micro-devices, made of 50/50, 27 kDa; 50/50, 40 kDa and 75/25 27 kDa PLGA, were prepared and characterized by in vitro and in vivo methods. The in vitro drug release from three micro-devices followed zero-order kinetics, and PLGA micro-device with the higher molecular weight and lactide/glycolide ratio tended to a longer sustained release period. The in vivo release results agreed with the in vitro results and drug release in vivo was faster than that in vitro for each of micro-devices. And three micro-devices showed different tumor inhibition effect in the tumor bearing mice. In addition, the SEM and weight loss experiments showed that PLGA micro-devices with lower molecular weight and lactide/glycolide ratio had faster degradation. These data provided the information for the optimization of the novel three-dimensional biodegradable micro-device to obtain more suitable systems for controlled release and to meet release requirements of different drugs.

Keywords Three-dimensional micro-device · Poly(lactic-*co*-glycolic acid) · 5-Fluorouracil · Microelectromechanical systems · Controlled drug release

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Introduction

Failure in the surgical treatment of cancer is often caused by cancer recurrence in the areas of surgical dissection. Inadequate resection margins and invasion of vital structures may increase the risk of cancer recurrence in patients with breast, lung, colon, rectal, and pancreatic malignancies (Jacquet et al. 1996). Systemic chemotherapy, though beneficial in treating cancer, is not sufficiently effective in controlling tumor recurrence after surgery due to the short biological half-life of the drug, lack of tissue specificity, and systemic toxicity (Seo et al. 2011; Nandagiri et al. 2011). Numerous studies have been conducted on drug delivery systems in which the drug is released constantly to maintain the required concentration and released locally to reduce systemic toxicity. Existing drug release systems include infusion pouch and osmotic pump (Manabea et al. 2004a), gelatinous matrix or gel (Manabea et al. 2004b; Ogura et al. 2006; Okinol et al. 2003), and polymer films (Liu et al. 2010; Park et al. 2010).

In our previous work, we have constructed a drug delivery system using microelectromechanical systems (MEMS) technology (Yang et al. 2005). The system is a three-dimensional biodegradable micro-device, in which poly(lactic-*co*-glycolic acid) (PLGA) is the carrier due to its biodegradation and biocompatibility (Fig. 1). The device is placed around the tumor site during the surgical procedure to perform local drug delivery, which can improve the therapeutic efficacy and minimize the systemic toxic effect. This three-dimensional device has a large array of micro-chambers, and different drugs with different physico-chemical properties can be filled in separately. The micro-chambers are flexible in shape and drug loading, which can be tailored easily and accurately to meet different clinical demands. The device is

biodegradable and there is no need for a surgical procedure for removal.

The objective of the present study was to investigate the effect of the molecular weight and monomer composition of PLGA on the drug release from the three-dimensional micro-device. 5-Fluorouracil (5-FU) was chosen as the model drug and the drug delivery was characterized by in vitro and in vivo release tests. The morphology of the micro-device was analyzed by scanning electron microscopy after the tests. The tumor inhibition effect of the device was evaluated by embedding it into the tumor bearing mice. The weight loss of the device was studied.

Experiments

Materials and animals

5-FU was obtained from Nantong Pharmaceutical Company (Jiangsu, China). PLGA (PLA/PGA ratio: 50/50, Mw: 40 kDa; 50/50, 27 kDa; 75/25, 27 kDa) was purchased from Daigang Biological Technology Co., Ltd. (Shandong, China). All other reagents were of HPLC or analytical grade.

Healthy male ICR mice weighing 18–20 g were obtained from the Laboratory Animal Center of Xi'an Jiaotong University and kept in a clean room at 25 °C with free access to water and food. Murine sarcoma S180 was obtained from the Laboratory Animal Center of the Fourth Military Medical University (Xi'an, China). The protocol of animal study was approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University.

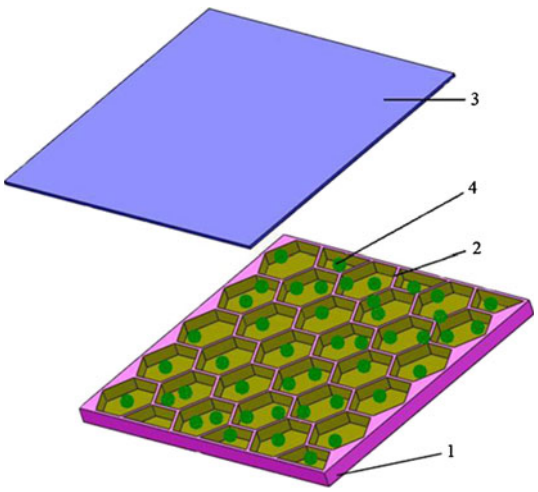

Fig. 1 A schematic diagram of PLGA micro-device. (*1*) PLGA matrix, (*2*) micro-chamber, (*3*) release membrane, (*4*) drug

Preparation of 5-FU micro-devices

5-FU micro-devices with honeycomb-structure were made of PLGA with different molecular weights and monomer compositions. Two grams of PLGA was dissolved in 16 ml acetone and poured on the polydimethylsiloxane molds. After being baked and cooled, the PLGA matrix was peeled from the molds. Fine powders of 5-FU were filled into the micro-chambers of the PLGA matrix using an injector, and a membrane made of the same PLGA material was sealed on the matrix. Altogether three PLGA micro-devices were constructed: 50/50, 40 kDa; 50/50, 27 kDa and 75/25 27 kDa.

In vitro drug release

The 5-FU loaded micro-devices with different PLGA formulations were placed in triangular flasks with 50 ml of phosphate buffered saline (PBS, pH 7.4) as release medium. The triangular flasks were incubated and shaken in an air bath with constant-temperature oscillator (THZ-82, Jiangsu Jintan Zhengji Instruments Co., Ltd.) at 37 °C with a speed of 40 ± 10 r/min. The release medium was replaced completely by fresh PBS and assayed at every sampling interval.

The concentration of 5-FU in the medium was determined by high performance liquid chromatography with a Shimadzu LC10ATvp pump and SPD-10AVP Plus UV–Vis detector (Kyoto, Japan). The chromatography was performed on a C18 column (5 μm, 150 mm × 4.6 mm) at the wavelength of 270 nm and the flow rate of 0.7 ml/min. The mobile phase consisted of methanol and purified water (5:95, v/v), and the injection volume was 10 μl.

In vivo drug release and antitumor efficacy of the micro-device

5×10^5 viable murine sarcoma S180 cells were inoculated at the left flank of the ICR mice. 3 days later, the mice were divided into four groups and received the following treatments separately: (1) control with no treatment ($n = 35$), (2) PLGA (50/50, 27 kDa) micro-device containing 7.2 mg 5-FU ($n = 35$), (3) PLGA (50/50, 40 kDa) micro-device containing 7.2 mg 5-FU ($n = 35$), and (4) PLGA (75/25, 27 kDa) micro-device containing 7.2 mg 5-FU ($n = 45$). Then, the mice were anaesthetized with diethyl ether. A 1 cm incision was made in the left dorsal area around the tumor and a 5-FU micro-device was embedded into the subcutaneous tissue. The incision was then sutured. At predetermined time points, five mice in each group were sacrificed. The tumor was excised and the micro-device was retrieved. The exercised tumor was

wiped with filter paper and weighed for evaluating the in vivo antitumor efficacy.

The retrieved micro-devices were dried under reduced pressure and dissolved in 0.5 ml chloroform by ultrasound for 30 min. The solution was then diluted to 100 ml with distilled water. After centrifugation at 12,000 rpm for 10 min, the supernatant was collected and determined by HPLC method. The in vivo cumulative release of 5-FU was calculated according to the residual 5-FU content in the retrieved PLGA micro-device.

In vivo and in vitro weight loss

In vivo weight loss of the PLGA micro-device was determined by weighing the device before it was embedded into the subcutaneous tissue and after it was retrieved at predetermined time points.

The micro-devices were put into the triangular flasks and treated the same way as in the in vitro release study. Samples of the devices were collected at predetermined time points and were weighed after drying for 24 h in a vacuum oven. The residual 5-FU content in the dried PLGA micro-device was determined using the method described in Section "In vitro drug release".

The weight loss in percentage was calculated according to the following equation:

$$\text{Weight loss } (\%) = \frac{(W_0 - W_{d0}) - (W_t - W_{dt})}{W_0 - W_{d0}} \times 100\,\%,$$

where W_0 is the initial weight of the micro-device in vivo/in vitro, W_{d0} is the initial weight of 5-FU, W_t is the weight of the micro-device at time point t, and W_{dt} is the weight of 5-FU at time point t.

Scanning electron microscopy

The structural changes of the micro-device with time were imaged by scanning electron microscopy (SEM). Micro-device samples from the in vitro and in vivo release tests were dried and sputtered under an argon atmosphere with gold. Then images of the morphologies on the cross-section of the samples were taken by JEOL JSM-840 scanning electron microscope (JEOL Ltd., Japan). The accelerating voltage used was 40 kV.

Statistical analysis

Results were expressed as mean ± standard deviation. Statistical comparison was made by one-way analysis of variance (AVONA) and $P < 0.05$ was considered statistically significant.

Results

Drug release

The profiles of the in vitro cumulative release of the PLGA micro-devices with three formulations are shown in Fig. 2a. The release profile of 75/25, 27 kDa PLGA micro-device was rather biphasic, with an approximate zero-order release followed by a plateau at the last few days of the experiment timescale. The release from 50/50 PLGA micro-devices fitted zero-order release kinetics well, and the correlation coefficient was 0.9942 for 27 kDa PLGA and 0.9909 for 40 kDa PLGA, respectively. The release rates of 50/50, 27 kDa, 50/50 40 kDa and 75/25, 27 kDa PLGA micro-devices were about 0.50, 0.35, and 0.21 mg/day, respectively, in zero-order release period. The 50/50, 27 kDa PLGA micro-device released 80 % of 5-FU in up to 11 days, while it took the 50/50, 40 kDa PLGA micro-device 14 days to release the same proportion of the drug. The 75/25, 27 kDa PLGA micro-device released less than 80 % of 5-FU in 30 days. These data indicated that the 50/50 PLGA micro-device with lower molecular weight had a relatively faster release rate compared with that with higher molecular weight, and that the drug was released faster from 50/50 PLGA micro-device than from 75/25 PLGA micro-device with the same molecular weight.

Figure 2b gives the profiles of the in vivo drug release. The micro-devices with three PLGA formulations showed similar zero-order release but different release rates in the tumor bearing mice. The 50/50, 27 kDa PLGA micro-device had the fastest rate, and released almost 100 % of the drug in up to 18 days. In contrast, the cumulative release amount from the 50/50, 40 kDa PLGA micro-device was 82.2 % on day 18, and that from the 75/25, 27 kDa PLGA micro-device was less than 80 % on day 24.

Antitumor efficacy

Figure 3 showed the variation of tumor weight with time after different treatments. In the control group, the tumor growth increased gradually and the mean tumor weight reached 5.6 g on day 15. As the overweight tumor caused difficulty in breathing and diet, and even death, the remaining mice were all sacrificed. In the group of mice treated with 50/50, 27 kDa PLGA micro-devices, the tumor weight increased from the beginning of the experiment to day 9, followed by a decrease until day 12, and then began to increase again. In the other two groups, 50/50, 40 kDa and 75/25, 27 kDa PLGA micro-devices showed a similar tumor inhibition effect, but the latter had a longer effect. The tumor weight increased and reached the summit on day 9 in both groups. It then began to decrease and the inhibition rate increased. On day 18, the mean tumor weight of

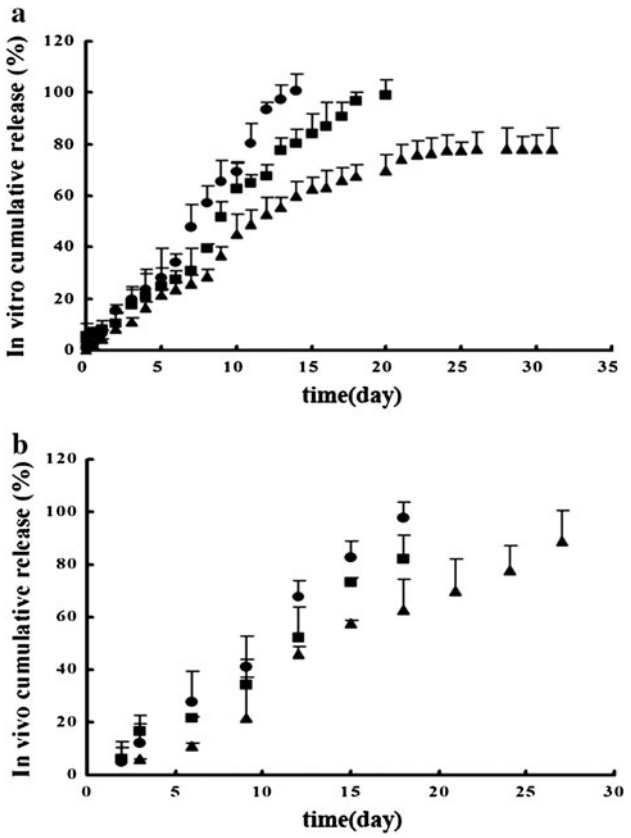

Fig. 2 The in vitro and in vivo cumulative release–time *curves* of 5-FU from the PLGA micro-device, **a** In vitro curve ($n = 3$), **b** in vivo curve ($n = 5$), (*filled circle*) 5-FU loaded 50/50, 27 kDa PLGA micro-devices, (*square*) 5-FU loaded 50/50, 40 kDa PLGA micro-devices, (*filled triangle*) 5-FU loaded 75/25, 27 kDa PLGA micro-devices. *Error bars* are expressed as standard deviation

the mice treated with 50/50, 40 kDa PLGA micro-devices dropped to 0.79 g, while that of the mice treated with 75/25, 27 kDa PLGA micro-devices fell to 1.22 g and continued falling to 0.29 g at the end of the experiment. There was a significant difference in the mean tumor weight on day 15 between the control group and the three treatment groups ($P < 0.05$).

SEM and weight changes of the micro-devices

The in vitro and in vivo morphologies of the 5-FU loaded PLGA micro-devices were characterized for the study of the drug release mechanisms of the devices. Figure 4 presents the SEM images of 50/50, 27 kDa PLGA micro-devices in vitro and in vivo on day 0, 3, 9, 15 and 27. There were initially no cracks or holes on the surface of the micro-devices. Tiny holes appeared 3 days later. The in vitro micro-devices had a comparatively greater porosity, whereas only the in vivo ones had creases. Then, pores became more in both in vitro and in vivo micro-devices, developing in the matrix as a result of hydrolysis of the matrix, fluid penetration, and simultaneous dissolution of the drug. On day 15, a sponge-like structure was seen in the micro-devices. The in vivo ones appeared to be multilayered. The outmost layer was flat; irregular voids and creases were seen in the inner layer; and deeper and larger creases could be seen clearly in the innermost layer. At the end of the experiment, the creases in vitro were obviously broader, while those in vivo were deeper.

Weight loss

Figure 5 showed the weight loss of the three PLGA micro-devices in the in vivo study. The weight loss of 50/50, 27 kDa PLGA and 50/50, 40 kDa PLGA micro-devices was detected on day 3 after implantation, and slightly increased to 3.70 and 2.96 %, respectively, on day 18. The former, though sharing the same weight loss profile with the latter, showed a faster rate. For 75/25, 27 kDa PLGA micro-devices, the weight loss was detected 6 days after implantation and reached 2.12 % till the end of the experiment and it was slower than that of 50/50 PLGA micro-devices.

Figure 6 gives the weight loss percentage of the three micro-devices at the end of the experiment. 50/50, 27 kDa PLGA had the largest weight loss both in vivo and in vitro, followed by 50/50 40 kDa PLGA and then 75/25, 27 kDa PLGA. There were significant differences between in vivo and in vitro weight loss percentages for all the three formulations ($P < 0.05$).

Discussion

Degradation and morphology

In the initial stage of PLGA degradation, water permeated into the micro-device, followed by swelling and expanding of the PLGA matrix. With further water uptake, matrix swelling and chain hydrolysis, the degradation entered a rapid stage, with most of the ester bonds fractured. Finally, the micro-device disintegrated gradually, with more pores, rifts, sinus and deep creases (Fig. 4) (Grayson et al. 2004). The difference in the weight loss rates of the three micro-devices might be explained by the different degradation rates affected by the ratio of lactide/glycolide in PLGA. It was found that 50/50 PLGA degraded faster than 75/25 PLGA probably because of its lower hydrophobicity (Toro et al. 2004). The results of this study also showed that the degradation of PLGA is also affected by its molecular weight and PLGA with lower molecular weight tends to degrade faster.

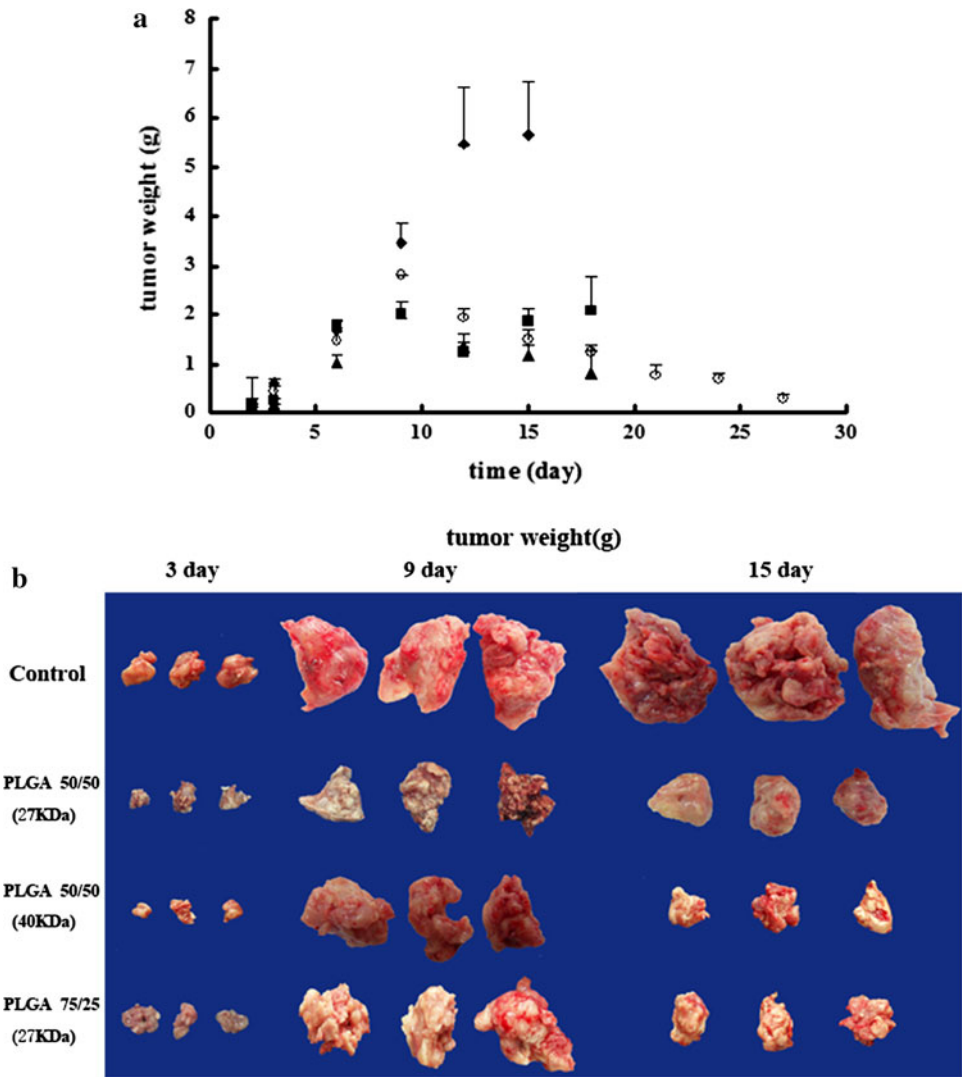

Fig. 3 Inhibitory effects of 5-FU from the PLGA micro-devices, **a** In vivo tumor weights of tumor bearing mice after different treatment. (*diamond*) Control group, (*square*) 5-FU loaded 50/50, 27 kDa PLGA micro-devices group, (*filled triangle*) 5-FU loaded 50/50, 40 kDa PLGA micro-devices group, (*circle*) 5-FU loaded 75/25, 27 kDa PLGA micro-devices group. *Error bars* are expressed as standard deviation ($n = 5$). **b** Representative photographs of S180 cell xenografts tumor in bearing tumor mice after different treatment on 3, 9 and 15 days

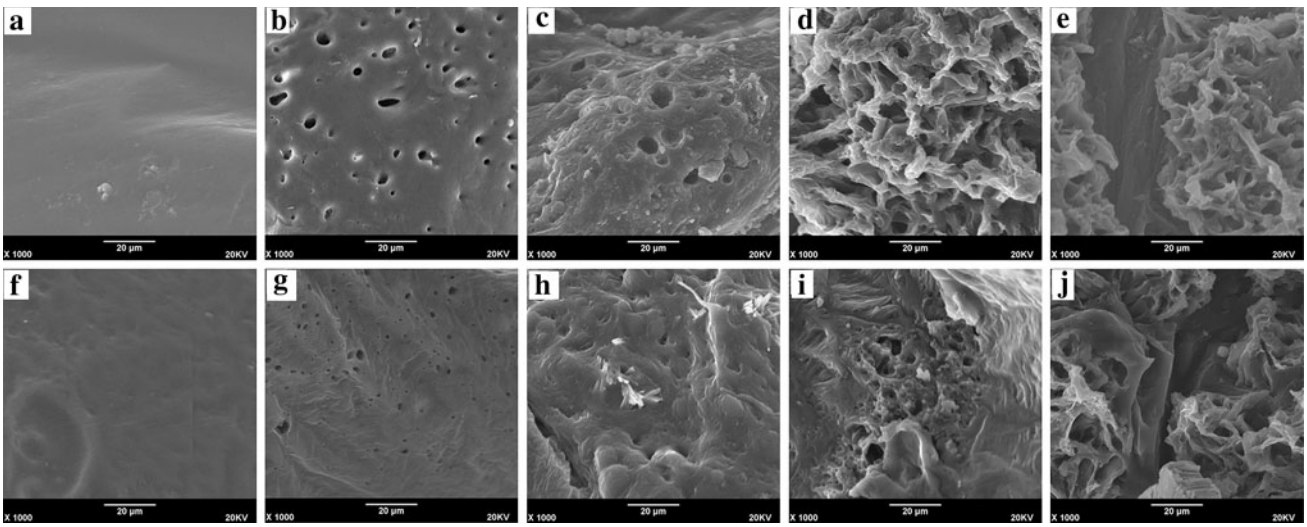

Fig. 4 SEM images of 5-FU-loaded micro-devices prepared with 75/25, 27 kDa PLGA. **a–e** represent the images of in vitro degradation on day 0, 3, 9, 15 and 27, respectively. **f–j** represent the images of in vivo degradation on day 0, 3, 9, 15 and 27, respectively

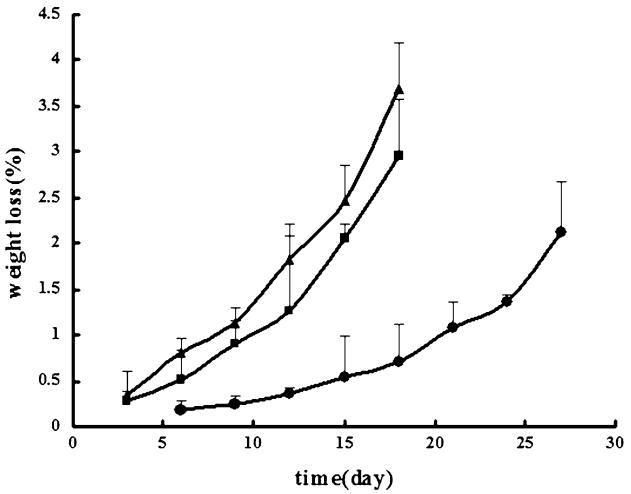

Fig. 5 In vivo weight loss profiles of PLGA micro-devices. (*filled triangle*) 50/50, 27 kDa PLGA, (*square*) 50/50, 40 kDa PLGA, (*filled circle*) 75/25, 27 kDa PLGA. *Error bars* are expressed as standard deviation ($n = 3$)

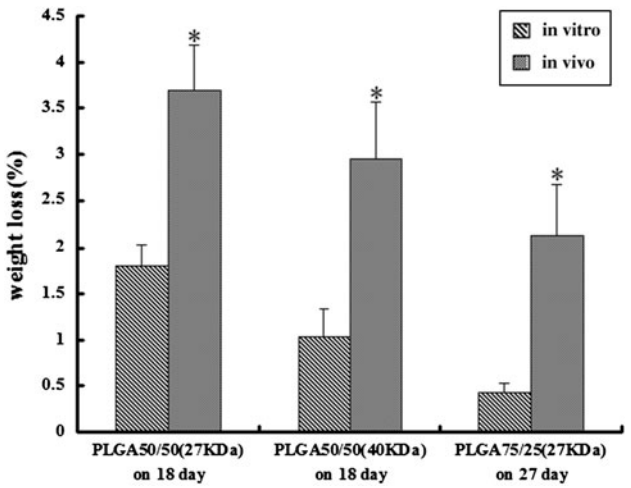

Fig. 6 Weight loss of PLGA micro-device of in vivo and in vitro. * $P < 0.05$ compared with in vitro. *Error bars* are expressed as standard deviation ($n = 3$)

For each of the three micro-devices, the weight loss in vitro was significantly lower than that in vivo, which corresponded well with previous studies (Tracy et al. 1999). The reasons for this might be that biological compounds in vivo could help water uptaken into the polymer (Anderson et al. 1997; Menei et al. 1993), and the foreign body response would facilitate the degradation of the polymer in vivo (Toro et al. 2004).

In vitro and in vivo drug release

Monomer composition and molecular weight are closely related to drug release. However, studies on the effects of the two factors on drug release are very limited. In the present study, these two parameters were varied and their influence on the drug release in vitro and in vivo was investigated. The results showed that PLGA micro-devices with higher molecular weight (40 kDa) had a lower drug release rate than that with lower molecular weight (27 kDa) both in vitro and in vivo. The in vivo results agreed well with the findings of Patel et al. (2010). It was also found that 50/50 PLGA micro-devices had a faster rate of drug release than 75/25 PLGA micro-device, which was in agreement with the finding in Lai et al. (2009). In addition, drug release in vivo was faster than that in vitro, which was consistent with previous studies (Meng et al. 2010; Patel et al. 2010).

Drugs formulated in polymeric devices are usually released by diffusion through the polymer barrier, degradation of the polymer materials, or a combination of both (Liu et al. 2011). The measurement of PLGA degradation showed that the drug release correlated well with the weight loss and the result of the SEM study. 50/50PLGA, which is more hydrophilic, had greater water absorption than 75/25 PLGA under the same condition (Toro et al. 2004). More swellings and hydrophilic channels occurred with more water influxing into the 50/50 PLGA matrix than the 75/25 PLGA matrix. As a result, a faster drug release from 50/50 PLGA micro-devices than 75/25 PLGA micro-device was achieved. In addition, the different degradation rates of PLGA micro-devices were possibly due to the difference between in vitro and in vivo environments. The acute inflammation, chronic inflammation and foreign body reaction after implantation might produce acid and other agents facilitating the biodegradation of PLGA (Ma et al. 2011), leading to widened holes and faster destruction (Fig. 4) which accelerated the release of 5-FU.

Antitumor efficacy

It was found that the concentration of 5-FU in tumor correlated well with the cumulative release in vivo in our previous study. The tumor in the mice treated with 50/50, 27 kDa PLGA micro-device was inhibited first but then grew again. This might be because 50/50, 27 kDa PLGA micro-device had the fastest drug release rate. The concentration of 5-FU in tumor rose quickly and tumor growth was inhibited, but it also decreased very fast and led to the increase of tumor weight after day 12. In contrast, 50/50, 40 kDa PLGA micro-device showed a better tumor inhibition effect after 9 days, which may be attributed to the longer steady release of 5-FU. PLGA (75/25, 27 kDa) micro-device had the lowest drug release rate. The tumor treated with this type of device was comparatively large at the beginning since the concentration of 5-FU might not be sufficient for tumor inhibition. With time going, the

sustained release resulted in increased 5-FU concentration which provided a better tumor inhibition effect.

In conclusion, a novel 5-FU loaded PLGA micro-device was prepared successfully, in which the drug release can be controlled by changing the size and shape of the micro-chambers. Different drugs can be filled in the micro-chambers separately. The future work will focus on investigating the efficacy of the micro-device loaded with combined drugs.

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Conflict of interest No any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations exists in submission of this manuscript. All authors alone are responsible for the content and writing of the manuscript.

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