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In vitro and *in vivo* evaluation of praziquantel loaded implants based on PEG/PCL blends

Liang Cheng, Lei Lei, Shengrong Guo*

School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

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ABSTRACT

In the present study, a series of praziquantel (PZQ) loaded implants based on PEG/PCL blends are fabricated by a combination of twin-screw mixing and hot-melt extrusion. *In vitro* drug release from these implants and the performance of the implants after implantation in rats are evaluated. XRD and DSC analysis results exhibit each component in the implants is mainly in its crystalline state. Dissolution test shows that the higher PEG content there is in the implants, the faster the drug will be released. Interestingly, PEG release from all implants is far faster than PZQ release, and complete PEG release occurs in 72 h. SEM result displays that after the *in vitro* drug release test, the cross-sections of implants with low PEG contents (0–5%) primarily consist of discrete pores; while those of implants with high PEG contents (10–30%) consist of interconnected pores or channels. The fitting results of drug release data with kinetic models reveal that PZQ release is governed by diffusion. After implantation, drug release becomes more moderate compared with *in vitro* drug release, and it tends to follow zero-order in the later stage. These results suggest that changing the composition of the PEG/PCL blends is an effective tool to adjust *in vitro*/*in vivo* drug release from the implants.

1. Introduction

Poly(ε-caprolactone) (PCL), an analogue of polyesters, has been approved by FDA as a biomedical material. Due to its excellent biocompatibility, longer degradation time than other polyesters (Dordunoo et al., 1997; Fialho et al., 2008), and high permeability to many drugs (Edlund and Albertsson, 2002; Sinha et al., 2004), PCL and its copolymers are becoming very attractive biomaterials in the design of various controlled/prolonged drug delivery systems (DDS), such as microspheres (Wang et al., 2008; Wang and Guo, 2008a,b; Zhang et al., 2009), micelles (Gong et al., 2009; Gou et al., 2009a), nanoparticles (Barbault-Foucher et al., 2002; Verger et al., 1998; Gou et al., 2009b; Wei et al., 2009) and implants (Carcaboso et al., 2008; Cheng et al., 2009; Lemmouchi et al., 1998). Moreover, with a low glass transition temperature (Tg, around −60 °C) and melting point (Tm, 60 °C), PCL is desirable for fabrication of DDS by some simple processing techniques such as melt molding, injection or extrusion without damaging the integrity of either the polymer or the drug molecule (Altpeter et al., 2004; Sprockel et al., 1997).

In our previous study, we prepared praziquantel (PZQ) loaded PCL implants by injection molding (Cheng et al., 2009). It was found that all the PCL implants could achieve sustained release of the loaded drug as long as 1 year. Nevertheless, one shortcoming of the PCL implants is that due to the strong hydrophobicity of PCL, drug release rate falls down quickly after an initial fast release of drug located on the surface of the implants, and this insufficient drug release may fail to reach the lower limit of the therapeutic window after implantation.

Poly(ethylene glycol) (PEG) is a non-toxic and hydrophilic polymer, which can be eliminated by glomerular filtration. Due to its capability to improve the wettability and solubility of water insoluble drugs, PEG has been extensively used as drug carrier (Law et al., 2004; Moneghini et al., 2001; Passerini et al., 2006) or drug release modifier (Herrmann et al., 2007a,b; Verhoeven et al., 2009). Additionally, with a relatively low melting point of 40–60 °C depending on the molecular weight, preparations based on PEG can also be conveniently fabricated by the existing melt processing techniques.

Much attention has been focused on the development and investigation of binary polymer blends, which allow the combination of desirable properties of different polymers with exceptional advantages over the development of novel polymeric materials. But, to our knowledge, few works have been done to develop a drug delivery device based on PEG/PCL polymer blends and investigate drug release behavior from the blends. Thus, one objective of this study is to prepare a series of PZQ-loaded implants using PEG/PCL blends of different composition as the carrier by hot-melt extrusion

* Corresponding author. Tel.: +86 21 34204793; fax: +86 21 34204793.
E-mail address: srguo@sjtu.edu.cn (S. Guo).

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and to evaluate the release behaviors of the loaded drug from the implants.

Hydatid disease caused by Echinococcus granulosus presents medical, veterinary, and economic problems worldwide (Anadol et al., 2001). PZQ is a broad-spectrum anthelmintic drug which has been recommended in the treatment of this disease (Moreno et al., 2001; Urrea-París et al., 1999). To date, PZQ is administrated to humans only by oral route. However, this approach has many disadvantages: (i) due to the extensive hepatic first-pass effect, more than 80% of drug is fast metabolized after absorption by gastrointestinal tract (Giorgi et al., 2001; Ridtitid et al., 2007; Sotelo and Jung, 1998); (ii) to achieve effective blood drug concentration (about 100 ng/ml), a large daily dosage and frequent administration are needed (Jung et al., 1997; Urrea-París et al., 2002); and (iii) a long-term treatment (one to several months) (Anadol et al., 2001) will lead to poor patient compliance.

Parenteral sustained-release formulation can be a desirable alternative to the oral preparation. Drug delivery by this route can avoid first hepatic metabolism and reduce the dose needed to be delivered. Moreover, sustained release of the drug will prolong treatment duration and reduce administration frequency. Consequently, the other objective of this study is to evaluate the performance of the PZQ-loaded implants after implantation in the subcutaneous tissue of rats, including *in vivo* drug release from the implants, drug concentrations in plasma, and tissue biocompatibility.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (PCL) (molecular weight, M_w = 50,000) was purchased from Daicel Polymer Ltd. (Minatoku, Tokyo, Japan). Praziquantel (PZQ) was obtained from Shanghai Jiachen Chemical Industry Co. Ltd. (Shanghai, China). Poly(ethylene glycol) (PEG) with a molecular weight of 1500 g/mol was obtained from Chinese Medicine Group (Shanghai, China). All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of implants

Implants were fabricated by fully blending PZQ particles with melting PCL and PEG and then extrusion. In brief, PCL and PEG were first fed into the hopper of HAAKE Rheocord System (Rheocord 90, HAAKE Mess-Technic GmbH, Germany) and heated until they were completely melted, well mixed. Afterward, PZQ was added slowly into the melt and mixed at 70 °C for 20 min at a stirring speed of 50 rpm. The resulting mixture was collected and further extruded using a lab-scale melt extruder at 70 °C. After cooling, the extruded rods with a diameter of 3 mm were cut into cylindrical implants with a length of 20 mm. The feed ratio of each implant batch was listed in Table 1.

2.3. Determination of drug contents in the implants

Ten implant samples from each implant batch were selected and weighed. Each implant was dissolved in 2 ml of acetonitrile, subsequently 8 ml of methanol were added to precipitate PCL. The resulting suspension was centrifuged at 10,000 × *g* for 15 min. 1 ml of the supernatant was withdrawn, filtered through a 0.45 μm pore size filter and analyzed by HPLC. The actual drug content in each implant batch was then calculated.

2.4. X-ray diffraction (XRD) analysis

XRD analysis for the implants were performed on a Rigaku D/max 2200 diffractometer with a Cu Kα radiation source at 40 kV voltage, 20 mA current. Before analysis, all implant samples were pressed into thin films on a Compression Molding Machine (XLB-D, Shanghai No. 1 Rubber Machine Factory) at ambient temperature. Then the films were placed in a steel holder and scanned over a 2θ range of 5–45° at a rate of 5°/min. Pure PZQ, PCL and PEG were directly loaded on a quartz sample holder and analyzed under the same condition.

2.5. Differential scanning calorimetry (DSC) analysis

Samples (4–7 mg) of pure PZQ, PCL, PEG and the implants were sealed in Al-crucible pans and measured by differential scanning calorimetry (DSC, Pyris 1, Perkin-Elmer, Inc., USA) at a scanning speed of 10 °C/min over a temperature range of 0–170 °C. Ultra-high pure nitrogen was used as the pure gas at a flow rate of 20 ml/min.

2.6. In vitro dissolution test

2.6.1. PZQ dissolution from the implants

In vitro PZQ release was carried out in triplicate in the release medium (0.2%, w/v sodium lauryl sulphate aqueous solution, SLS solution) under perfect sink condition. Each implant sample was immersed in 30 ml of SLS solution in a watertight vial and shaken at 37 °C in a water bath at 80 rpm. At predetermined time points, the release medium was completely taken out and replaced with 30 ml of fresh medium. PZQ concentration in release medium was determined by HPLC. The column was a Diamonsil C18 (particle size 5 μm, 4.6 mm × 250 mm) column. The mobile phase consisted of methanol:water (100:40, v/v). The flow rate was maintained at 1 ml/min and UV-detection was performed at 263 nm. The linear range of drug concentration was 1–200 μg/ml with a relevant coefficient of 0.9999.

2.6.2. PEG release from the implants

In vitro PEG release test was performed under the same condition as *in vitro* PZQ release test. The mass of each implant was measured at the beginning of the release test. At different time points, implants were taken out from the release solution and PZQ

Table 1
PZQ contents in the fabricated implants (n = 10).

Implant	Feed ratios of implant			Drug content[a] (%, w/w)	Relative drug content[b] (% ±SD)
	PZQ	PEG	PCL		
F1	50	0	50	50	99.8 ± 1.2
F2	50	5	45	50	101.4 ± 0.6
F3	50	10	40	50	99.6 ± 2.8
F4	50	20	30	50	100.6 ± 3.2
F5	50	30	20	50	98.8 ± 2.4

[a] Calculated by the feed ratios.
[b] Calculated by the formula as follows: relative drug content (%) = the determined drug amount in fabricated implant/the fed drug amount × 100%.

concentration in the solution was analyzed. After vacuum drying for 48 h, the weight of each implant was measured again and the cumulative release percentage of PEG over time was calculated by equation as follows:

$$\text{PEG released (\%)} = \frac{\text{Initial implant weight} - \text{final implant weight} - \text{amount of PZQ released}}{\text{Initial amount of PEG in implant}} \times 100\%$$

2.7. Scanning electron microscope (SEM)

At different time points, the morphologies (surface and cross-section) of the implants were imaged by scanning electron microscope (JSM-7401F, JEOL, Japan) operating at a voltage of 1 kV. The cross-section of the implant was obtained by first immersing the implant in liquid nitrogen for 10 min and then breaking off it with a scalpel. Prior to observation, all samples were placed on metal sample holders and sputter coated (Emitech K-575 Sputter Coater) with a gold–palladium target for 60 s at 20 mA.

2.8. Gel permeation chromatography (GPC)

Molecular weights of PCL were measured using GPC equipped with a refractive index detector (Waters, Model 1515, Mildford). Samples were dissolved in THF and eluted at a flow rate of 1 ml/min. Polystyrene standards (M_w ranges from 1.31×10^3 to 1.97×10^5 Da) (Tosoh Corporation, Japan) were used to obtain a primary calibration curve.

2.9. In vivo studies of PZQ-loaded implants

2.9.1. Subcutaneous implantation procedure

The experimental protocol was approved by the University Ethics Committee (Shanghai Jiao Tong University) for the use of experimental animals and complied with the guideline for Care and Use of Laboratory Animals. Twenty-eight Wistar-derived male rats weighing approximately 350 g were used. Animals were randomly divided into seven groups. Prior to implantation, implants were sterilized under UV light for 3 h. The implantation procedure is as follows: firstly, all rats were anesthetized by intraperitoneal injection of ethyl aminoformate aqueous solution (20%, w/v), then three separate 1 cm incisions were made at the left, median, and right planes of the dorsal area of the rat. Subsequently, three rods were implanted into the subcutaneous tissue, respectively, and the incisions were sutured. After the implantation, all animals were placed in a heated recovery chamber until conscious.

2.9.2. Determination of PZQ concentration in plasma

PZQ concentration in plasma was determined using a simple, sensitive HPLC method (Hanpitakpong et al., 2004). In brief, to 1 ml plasma sample, 6 μl internal standard working solution (diazepam, 100 ng/μl) was added. The plasma samples were vortexed for 5 s and extracted with 6 ml methyl-tert-butylether/dichloromethane mixture (2:1, v/v). After being subjected to mechanical tumbling for 30 min, the organic layer was separated through centrifugation at 10,000 × *g* for 15 min. The upper organic layer was transferred to a clean tube and evaporated to dryness under a stream of nitrogen at 45 °C. The residual was reconstituted in 200 μl of mobile phase and 100 μl of the resultant solution was injected into the HPLC system. The chromatograph conditions were nearly the same as those for *in vitro* PZQ analysis except the wavelength, 217 nm was chosen to determine PZQ in plasma. The extraction efficiency of PZQ from plasma was greater than 90% and the precision of the assay was <15%.

2.9.3. In vivo cumulative release of PZQ and PEG

The animals were sacrificed at different time points and the implants were retrieved from subcutaneous tissue. After rinsing and drying the implants, the amount of residual PZQ in the implant was determined according to the method described in Section 2.3. *In vivo* cumulative release percentage of PZQ was calculated as follows:

$$\text{PZQ release percent (\%)} = \frac{\text{Initial PZQ amount} - \text{residual PZQ amount}}{\text{Initial PZQ amount}} \times 100\%$$

The calculation of *in vivo* cumulative release percentage of PEG was done according to the same method as that detailed in Section 2.6.2.

2.9.4. Histological analysis

Tissue samples from the implantation sites were excised, rinsed by distilled water and placed in 10% neutral buffer formalin. Hematoxylin and eosin (H&E) staining was used to characterize and quantify the inflammation-mediating cells in the vicinity of the implants in response to the inflammation induced by tissue injury on implantation and by the continued presence of the implants.

3. Results and discussion

3.1. Characterization of the PZQ-loaded implants

3.1.1. The appearance and microstructures of the implants

In this study, both PCL and PEG function as the matrix material for the implants. PZQ, which appears as a prismatic crystal (Fig. 1a), is the active substance. Drug particles with a length of less than 20 μm are used. Generally, the minimum requirement for a successful extrusion process is that the extrudates have a good external appearance. As shown in Fig. 1b, the extruded implants are smooth and compact by visual observation. Furthermore, at a high magnification (1000×), both the surfaces and cross-sections of the implants are very dense (Fig. 1c and d). This suggests that the present production condition is suitable for the fabrication of the implant.

3.1.2. Content uniformity

A potential shortcoming associated with hot-melt extrusion (HME) is the poor drug dispersion in polymer matrix due to short mixing time. To preclude this defect, a combination of twin-screw mixing and HME is adopted to fabricate the implants. Immediately after the fabrication processes, PZQ contents in the implants are determined by HPLC. As listed in Table 1, the values of relative drug content are quite close to 100 and the calculated standard deviation (SD) values are very small for all implants, suggesting that PZQ is dispersed homogeneously in the polymer matrix. Moreover, there is no additional peak observed in all chromatography traces, revealing that PZQ is stable during the fabrication processes involving heat and shearing force.

3.1.3. XRD analysis

The XRD patterns of PZQ, PCL, PEG and all implants are shown in Fig. 2. PZQ is crystalline, as demonstrated by sharp and intense diffraction peaks at 6.22°, 7.92° and a series of peaks between 10° and 25°. The XRD pattern of PZQ is consistent with that of racemic PZQ crystal, as reported by Liu et al. (2004). PCL displays two characteristic peaks at 21.24° and 23.52°, confirming its semi-crystalline structure (Wang et al., 2008). PEG has two distinct peaks at 19.24° and 23.42°. For all implants, there exit obvious characteristic peaks corresponding to PCL and PZQ, manifesting PCL and PZQ are mainly in crystalline state. However, it is difficult to directly identify the

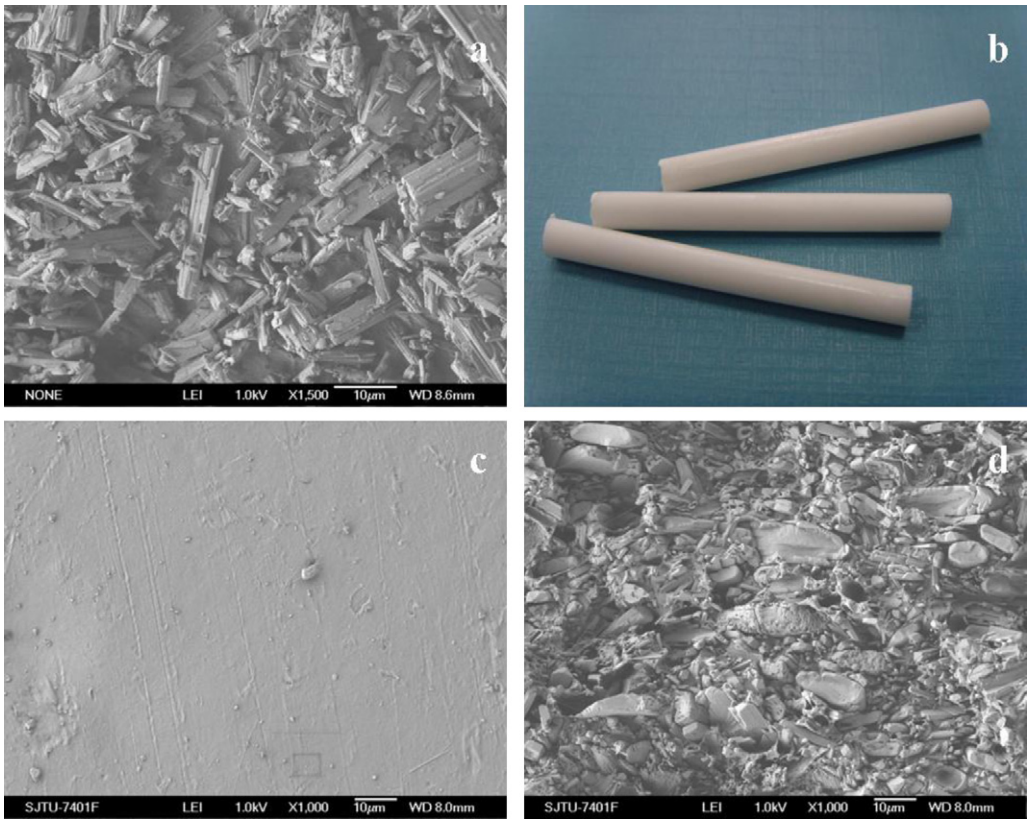

Fig. 1. PZQ particles and implants: (a) SEM image of PZQ particles; (b) outward appearance of the fabricated implants, taken by digital camera; (c) SEM image of the representative surface of the implants; and (d) SEM image of the representative cross-section of the implants.

characteristic peaks of PEG, since its crystalline peaks are overlapped with some peaks of PCL and PZQ. By comparing the change in diffraction peak intensity at 19.22° and 19.24° (indicated by an arrow), it is possible for us to preliminarily distinguish physical state of PEG in the implants. It should be noted that peak intensity at the two diffraction degrees is nearly equal for PEG free implant, but with the elevation of PEG content peak intensity at 19.24° increases continuously. Furthermore, when PEG content rises up to 10%, it is surprising that the two peaks are integrated into a single peak. We conclude that this phenomenon should be ascribed to the crystalline PEG. Accordingly, PEG is also in its crystalline state in all implants.

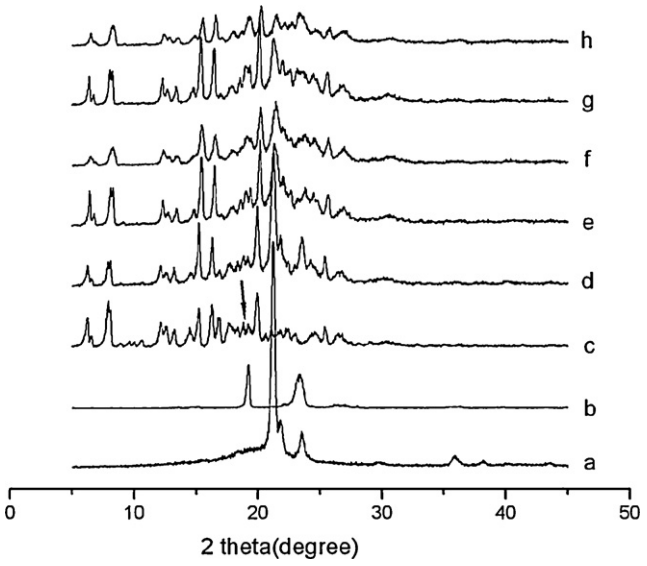

Fig. 2. X-ray diffraction patterns of pure PCL (a), pure PEG (b), pure PZQ (c) and implants with different PEG contents (0, 5, 10, 20 and 30%), respectively (d, e, f, g and h).

3.1.4. DSC analysis

To further explore the physical state of each component in the implants, thermal behaviors of PCL, PZQ, PEG and drug-loaded implants are analyzed by DSC and all thermographs are presented in Fig. 3. The DSC trace of PCL exhibits a broad melting peak between 40 and 70 °C, centered at about 62 °C. The DSC traces of PZQ and PEG show a sharp endothermic peak at 143.7 and 49.6 °C, respectively. For implant F1, DSC trace displays two endothermic peaks at 60 and 127.4 °C. The former should be the melting peak of PCL, while the latter should be the melting peak of incorporated PZQ. The depressed melting point for PZQ implies that a part of drug is dissolved into the melting PCL during the fabrication processes. DSC trace of implant F2 is nearly identical to that of implant F1 (not shown). However, for implant F3, besides the melting peaks corresponding to PCL and PZQ, an additional endothermic peak at 45.3 °C emerges. Moreover, with the steady increase in PEG content, the area of that peak at the same temperature continuously increases, whereas those of peaks for PCL continuously decrease. These results suggest that the endotherm peak at 45.3 °C must be the melting peak of PEG. Despite the dissolution of a part of PZQ in PCL phase, PZQ, PEG and PCL are primarily in their crystalline state in the implants.

3.2. In vitro release test

3.2.1. PZQ release from the PCL implants

Initially, PZQ release from PCL matrix without PEG addition is investigated. As shown in Fig. 4, it is clear that drug release can be

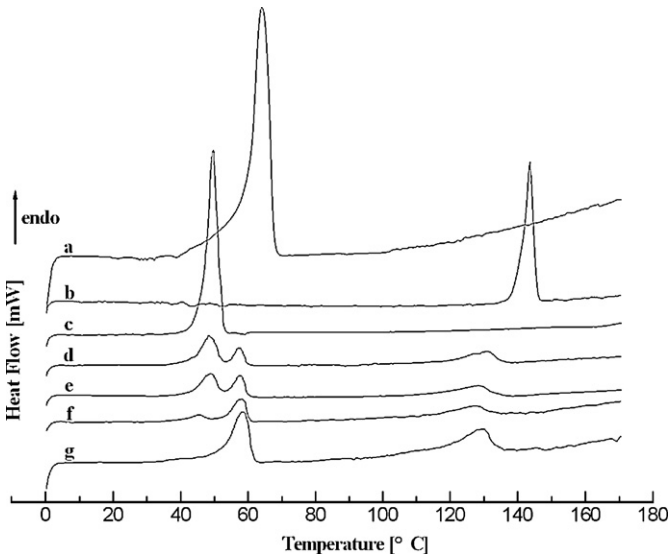

Fig. 3. DSC curves of pure PCL (a), pure PZQ (b), pure PEG (c) and implants with different PEG contents (30, 20, 10 and 0%), respectively (d, e, f and g).

divided into two stages, i.e. initial fast release stage and subsequent sustained-release stage. Approximately 10% of drug is released at day 1, subsequently the drug release rate gradually slows down, and till day 88, near 80% of the incorporated drug is released. Even that the PCL implant can release the drug in a sustained-release way, the slow release rate, especially in the later period of the dissolution test, may fail to reach the effective concentration after implantation. In order to improve the dissolution of PZQ, blends consisting of PCL and PEG at different ratios are used as the controlled-release matrix. It is noted that incorporation of 5% hydrophilic PEG into PCL matrix results in an obvious change in the drug release. Implant F2 releases 14% of drug at day 1 and 80% of drug within 70 days. Further increasing the PEG content, PZQ release is continuously accelerated. Importantly, not only the slope of the release curves, but also their shape is altered. For instance, implant F5 releases all loaded drug in less than 25 days.

To explore whether there exist significant differences between the release profiles after the incorporation of different amounts of hydrophilic PEG, f_2 (similarity factor) is introduced for the comparison (Moore and Flanner, 1996).

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} W_t (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where R_t is the reference drug release content at time point t, T_t is the test drug release content at time point t, n is the number of the sampling points, and W_t is an optional weight factors. Since all the release time points are treated equally, W_t is taken as 1. According to the guidance recommended by FDA, generally, two release profiles may be regarded as equivalence when the f_2 value is greater than 50. According to the calculated values of similarity factor (listed in Table 2), it is found that: (1) incorporation of a small amount of PEG (5%) into PCL implants can facilitate the dissolution of PZQ to some extent, but it cannot significantly increase the release rate; (2) when PEG content rises up to 10%, a significant acceleration on the drug dissolution can be achieved; and (3) drug release from the PCL implant can be further accelerated when elevating the PEG content up to 30%. These results suggest that the addition of hydrophilic PEG is very efficient to adjust PZQ release from the PCL implants.

Table 2
Values of similarity factor (f_2) between the release profiles of the implants containing different amounts of PEG.

Pairs of profiles	Values of similarity factor
$f_{2(F1–F2)}$	51.3
$f_{2(F1–F3)}$	37.7
$f_{2(F2–F3)}$	48.5
$f_{2(F2–F4)}$	46.4
$f_{2(F2–F5)}$	40.6
$f_{2(F3–F4)}$	83.1
$f_{2(F3–F5)}$	49.2
$f_{2(F4–F5)}$	51.9

3.2.2. PEG release from the PCL implants

It is evident that PEG plays an important role in controlling PZQ release, thus *in vitro* dissolution of PEG from the implants is also investigated. Fig. 5 exhibits the dissolution profiles of PEG from implants F3–F5. It is clear that release rate of PEG increases with the rising PEG content. Within 24 h, approximately 90% of PEG is released from implant F4 and F5, while 60% of PEG is released from implant F3. After 72 h, the whole incorporated PEG is released into

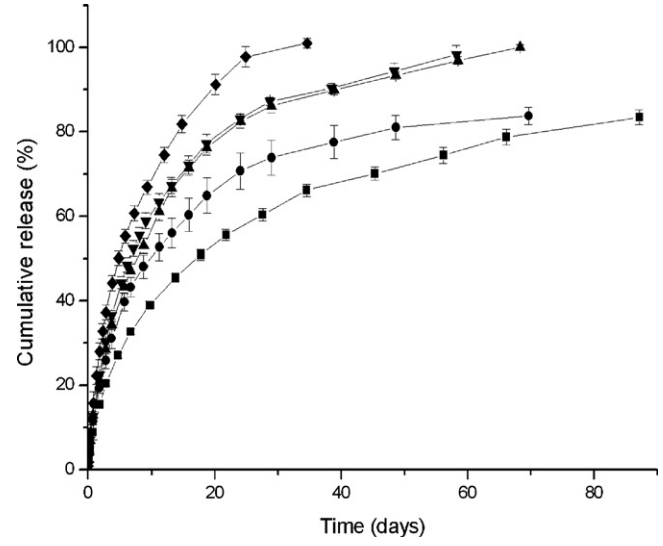

Fig. 4. *In vitro* release profiles of PZQ from implants: F1 (♦), F2 (■), F3 (●), F4 (▲) and F5 (▼) in 0.2% SLS aqueous solutions at 37 °C ($n = 3$).

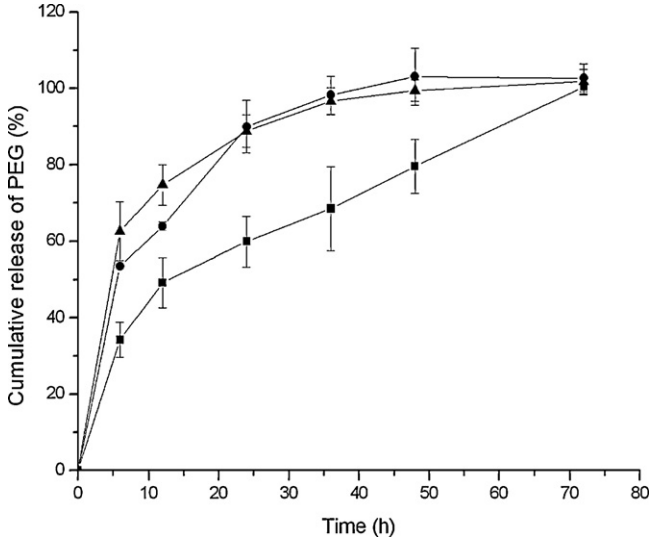

Fig. 5. *In vitro* release profiles of PEG from implants: F3 (■), F4 (▲) and F5 (●) in 0.2% SLS aqueous solutions at 37 °C ($n = 5$).

the release medium for all implants. Compared with PZQ dissolution profiles, PEG release at each time points is significantly faster than PZQ release ($p < 0.01$). Till the complete dissolution of PEG, only a small part of PZQ (about 30%) is released from the implants. As a result, it is reasonable to conclude that the voids formed by the rapidly released PEG essentially promote PZQ release from the implants.

3.2.3. Drug release mechanism

The release of either hydrophilic or hydrophobic drug from PCL based devices is primarily dominated by diffusion pattern (Rosenberg et al., 2007, 2008). The data of all *in vitro* release profiles are fitted by the zero-order, first-order and Higuchi equation, respectively. As listed in Table 3, the best fit is obtained with the Higuchi square root model ($r > 0.99$), indicating that drug diffusion is the predominant release mechanism for all implants. Interestingly, the value of the release constant (K_H) has a direct relationship with the contents of PEG in the implant, i.e. the higher the PEG content there is in the implant, the larger the value of the release constant is. This result demonstrates again that the incorporation of PEG can facilitate PZQ dissolution from these PCL implants.

Table 3
Data from regression fitting between dissolution profiles of PZQ and several kinetic models (zero-order, first-order, Higuchi). The fitting between the different models and the dissolution profiles is expressed via the correlation coefficient *r*.

Implant	Zero-order	First-order	Higuchi	
	r	*r*	K_H	*r*
F1	0.8969	0.6730	12.3461	0.9970
F2	0.8370	0.6372	16.4039	0.9961
F3	0.8587	0.6532	19.6540	0.9996
F4	0.8530	0.6407	21.0890	0.9993
F5	0.8983	0.6530	25.0889	0.9989

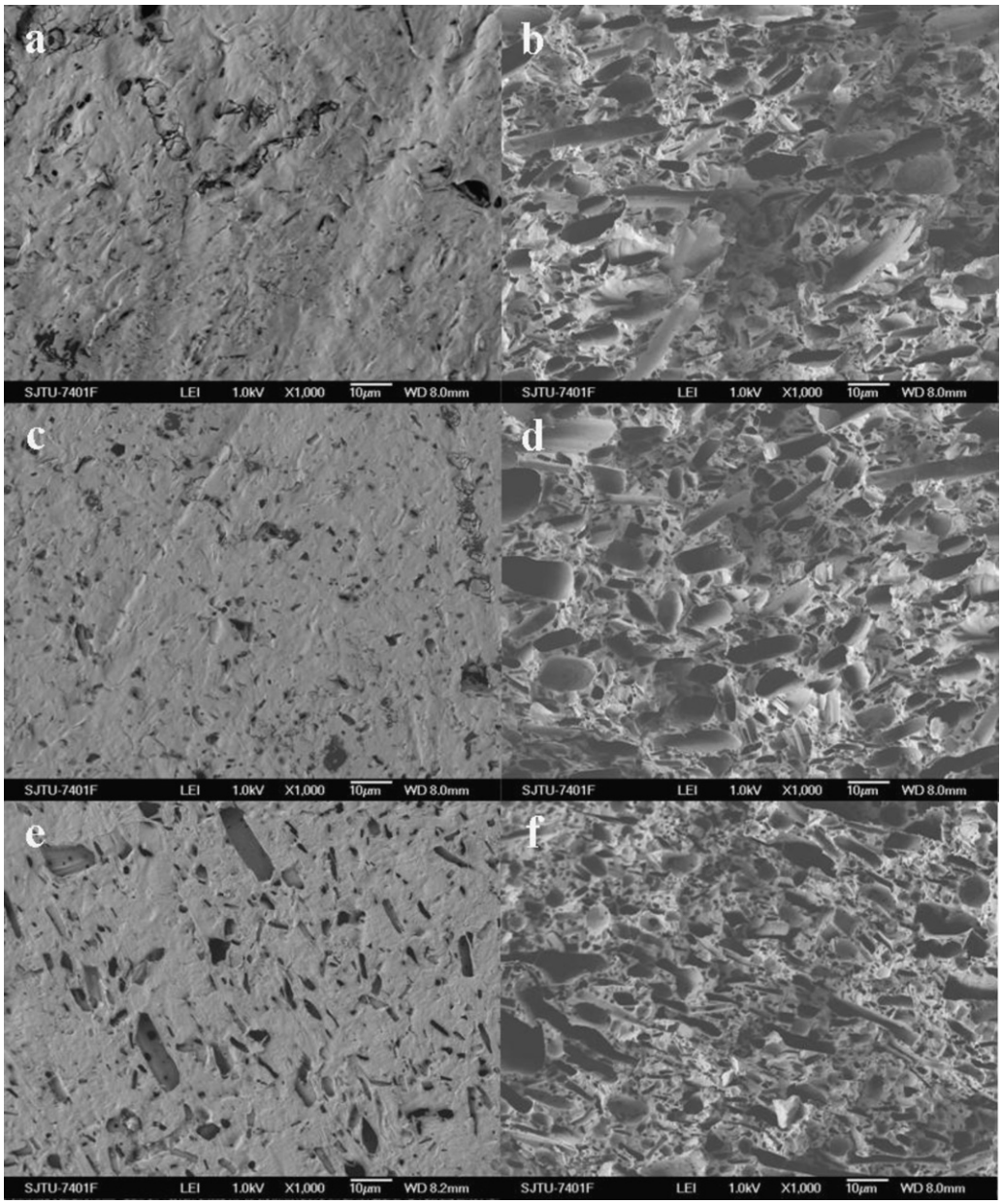

Fig. 6. SEM images: the surfaces of implants F1–F5, respectively (a, c, e, i and k); the cross-sections of implants F1–F5, respectively (b, d, f, j and l); the cross-sections of implant F1 and F3 at a magnification of 2000×, respectively (g and h); and the representative cross-section of F4 and F5 implant after incubation in release medium for 48 h (m).

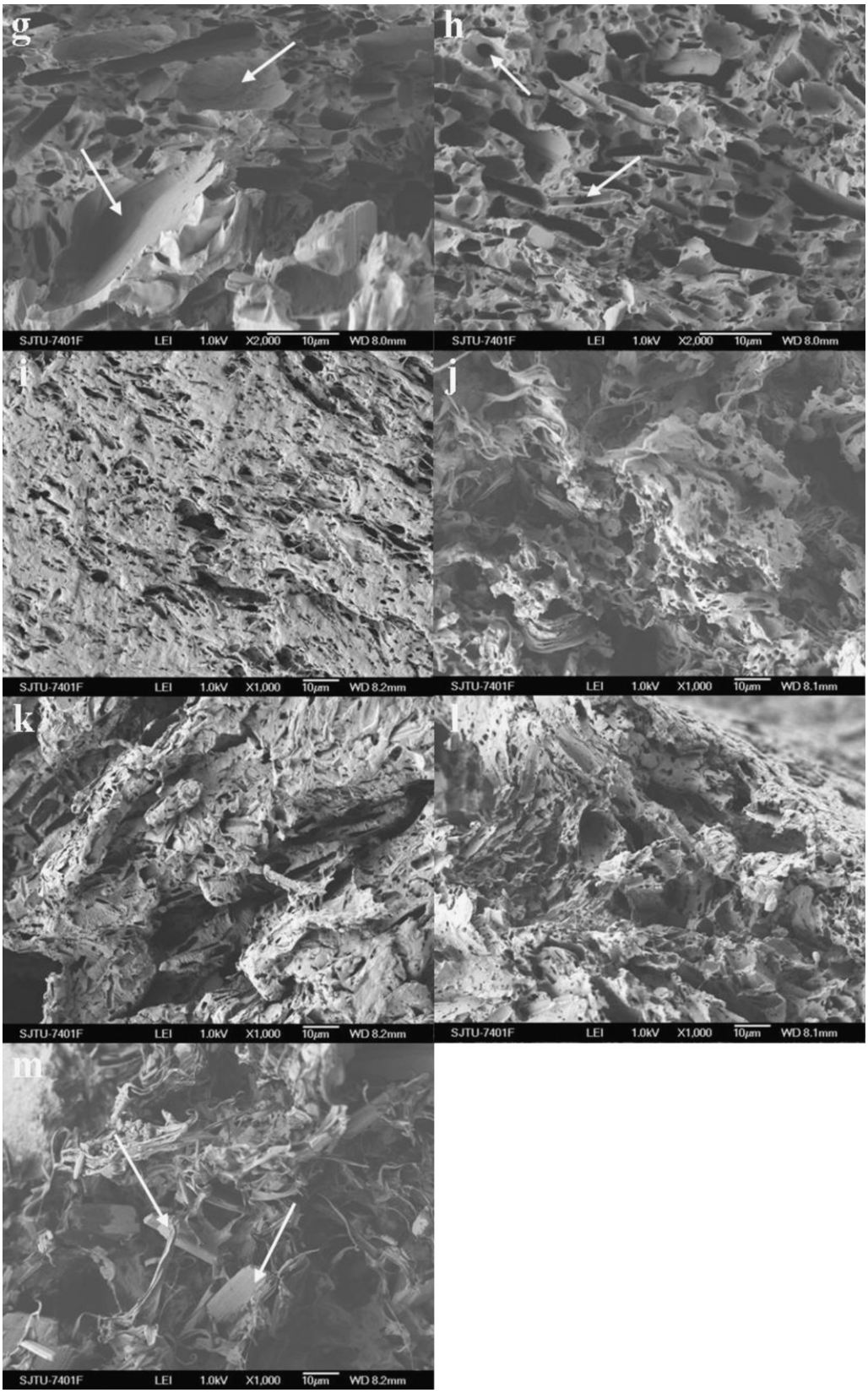

Fig. 6. (*Continued*).

Generally, pores, created by the dissolution of a water soluble additive, could shorten the diffusion distance of drug, thus accelerating drug release. In this study, varying contents of PEG are encapsulated in the implants, and in implant F5 the content of PEG is higher than that of PCL, thus the diffusion route of PZQ from the implants may be different. To elucidate the underlying diffusion mechanism of PZQ, the morphologies (surface and cross-section) of all implants are observed by SEM after *in vitro* release

test. As presented in Fig. 6a, the surface of implant F1 is still very dense, only with a little roughness, indicating drug particles on the surface are completely encapsulated by PCL material after fabrication. Compared with the unreleased cross-sections, many discrete pores with regular shape are observed in the cross-sections of implant F1, and the appearance of these separate pores can be ascribed to the dissolution of drug crystals. For implant F2, the surface and cross-section morphologies are nearly identical to those of implant F1, only a little more pores are observed for implant F2 (Fig. 6c and d). As for implant F3, many pores are formed on the surface of the implant and the shape of these pores is similar to the prismatic drug crystals (Fig. 6e), implying that PEG can play a certain role in the encapsulation of drug particles as PCL does. Additionally, in the cross-section the remaining pores are not as intact as that of implant F1 and F2, and some cavities are interconnected by the pores left by released PEG (denoted by the white arrows) (Fig. 6h). With respect to implant F4 and F5, both the surface and the cross-section morphologies are apparently different from those of above-mentioned implants. As exhibited in Fig. 6i and k, a rough and porous surface is presented for implant F4 and F5, even some degree of erosion occurs on the surface of implant F5. As for the cross-sections, it is difficult to find any intact pores with a shape similar to the drug particles and the remainder of the implant presents a complex network structure which consists of many interconnected large channels (Fig. 6j and l). This reveals that in the case of implant F4 and F5, PEG particles probably exist as a consecutive phase in the implant. To verify our hypothesis, the cross-sections of implant F4 and F5 incubated in the release medium for 48 h are observed (in this time interval PEG has been completely released from the implants, while the drug has not). As shown in Fig. 6m, the same interconnected channels are generated in the representative cross-section of the two implants and some unreleased drug particles are exposed (denoted by the white arrows). Based on these results, we may make a conclusion that drug release from implant F1 and F2 is primarily by slow diffusion through PCL matrix and/or the pores created by the released drug particles; while drug release from implant F3 is mainly governed by diffusion through interconnected pores; as for implant F4 and F5 drug release is predominated by diffusion through the channels formed by the fast released PEG.

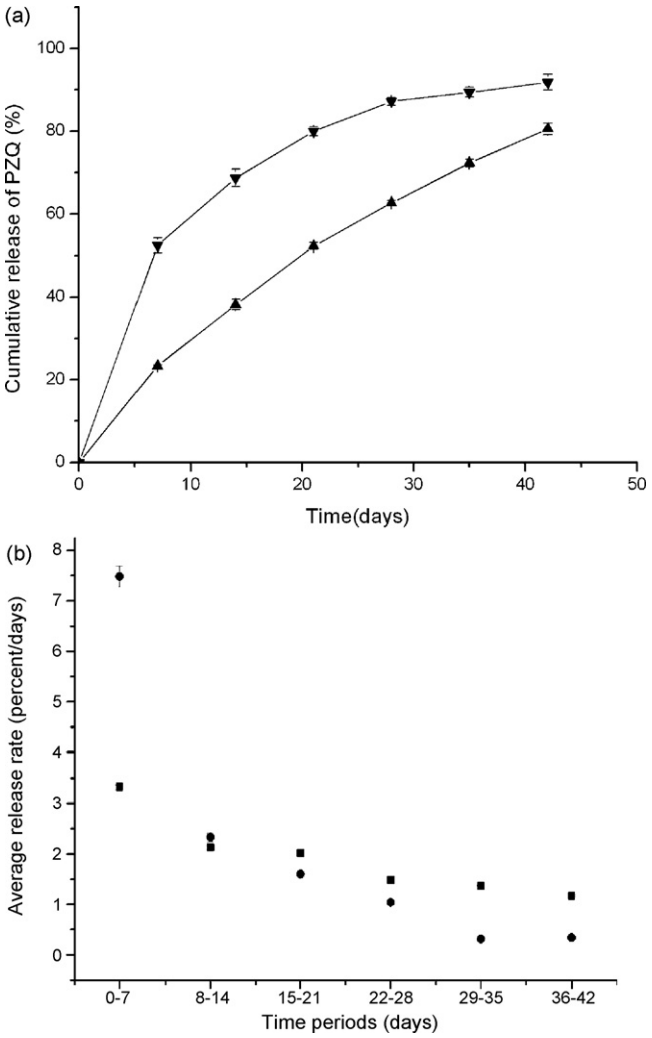

Fig. 7. PZQ release from implant F4: (a) *in vitro* (▼) and *in vivo* (▲) cumulative release curve; (b) *in vitro* (●) and *in vivo* (■) average drug release rate (percentage per day).

3.3. *In vivo evaluation*

A sustained-release implant which could extend the *in vivo* drug release duration as long as at least 1 month is desirable for the treatment of hydatid disease. According to the *in vitro* release profiles, it seems that with a proper release rate, implant F4 is the most promising candidate. Thus, *in vivo* release behavior of implant F4 is investigated in the present study.

3.3.1. *PZQ release from the implant*

At various time intervals, a group of animals are sacrificed and the unreleased drug in the implant is determined by HPLC. As illustrated in Fig. 7a, it is notable that *in vivo* drug release is slower than *in vitro* release during the experiment period. In the first week, the implant releases only 23.1% of drug *in vivo*, while 52.4% *in vitro*. Till 7 weeks, approximately 80.5% of drug is released from the implant *in vivo*, while more than 90% of drug is depleted *in vitro*. However, *in vivo* drug release rate is higher than *in vitro* release rate after the initial burst release in the first week (as shown in Fig. 7b). The average percentage of drug released *in vivo* per day is always maintained at a high level of 1.5–2.5% during the subsequent 5 weeks, while the average percentage of drug released *in vitro* per day quickly drops below a level of 1% after 3 weeks. Moreover, when fitting the data of *in vivo* release profile except the first data point by several kinetics models (zero-order, first-order and Higuchi equation), it is interesting that drug release follows a near zero-order release kinetics model well ($r = 0.9930$). This release kinetics is expected in that constant drug release will generate stable drug concentration in blood.

3.3.2. *PEG release from the implant*

In vivo PEG release from the implant is also investigated according to the same method as described in Section 2.6.2. Prior to the calculation of PEG release percentage, the *in vivo* degradation of PCL is evaluated by GPC. The weight average molecular weight of raw PCL is 5.004×10^4, while the determined value after 6-week implantation is 5.157×10^4. The highly consistent molecular weights demonstrate that PCL stays in its original state under physiological environment during the entire experimental period. As a result, the weight loss method is also feasible for the determination of *in vivo* PEG release. Compared with the *in vitro* release test, *in vivo* PEG release becomes slower but still significantly faster than *in vivo* drug release. In the first week, about 81.7% of PEG is released from the implant, and in the following week the incorporated PEG is completely released from the implant. The relatively slow *in vivo* release of PEG may account for the more moderate *in vivo* release of drug.

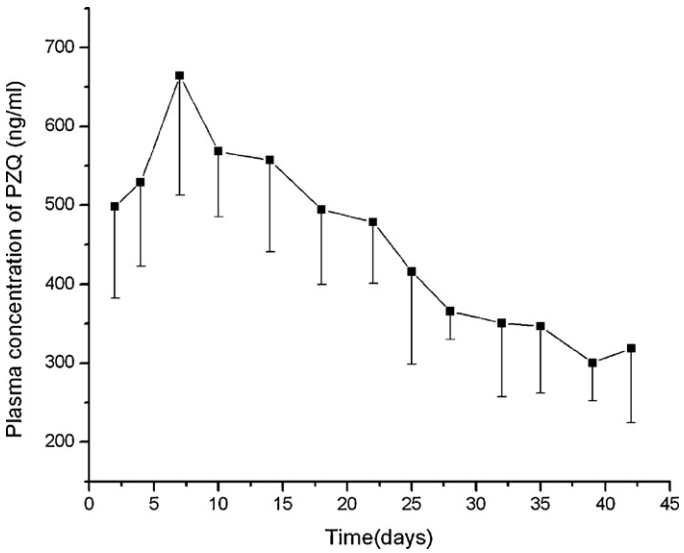

Fig. 8. PZQ concentrations in plasma versus time ($n = 4$).

3.3.3. PZQ concentration in plasma

Fig. 8 plots the drug concentrations in rat plasma as a function of time after subcutaneous implantation. It is obvious that the drug concentrations drop slowly as time progresses and always stay in a narrow range of 300–700 ng/ml. In the first week, all plasma drug concentrations are above 500 ng/ml and the maximum drug concentration of about 670 ng/ml is attained at day 7. Afterward, the drug concentration slowly falls off, but it always stays above a level of 300 ng/ml during the entire experimental period. According to previous studies, when PZQ concentration in plasma is above 100 ng/ml, it could exert an excellent anti-parasitic effect (Jung et al., 1997; Sotelo and Jung, 1998). As a result, the implant evaluated in this study exhibits a promising prospect to treat hydatid disease.

3.3.4. Histological evaluation

Excessive and chronic inflammation is a known factor contributing to implant failure (Patil et al., 2007), as such, it is an important variable to be considered in an evaluation of tissue response to biomaterial implants. The histological photograph of normal rat subcutaneous tissue (Fig. 9a) illustrates the presence of abundant hair follicles, associated microvascular and muscle segments, as well as a predominance of dense connective tissue with sparse immunocytes. By contrast, subcutaneous tissue excised at 1 week exhibits a severe humoral and cellular immunogenic response that is characterized by expansion of microvascular surrounding the implant site and massive infiltration of inflammatory cells, specifically neutrophils. The neutrophils are followed by macrophages, monocytes, fibroblasts and eventually giant cells form. The inflammation-mediating cells in the implant site during the acute phase of inflammation are stained purple using H&E, while normal tissue stained pink (Fig. 9b). Prolonged *in vivo* residence of the implant causes the acute inflammation reaction to evolve into the chronic phase, which is characterized by the deposition of fibrotic tissue around the implant and a decrease in the number of inflammatory cells (Fig. 9c). By week 6, the implants are completely encapsulated within a fibrotic capsule, which is predominantly composed of mature collagen deposition, associated with the presence of fibroblasts, blood vessels and minimal inflammation cells (Fig. 9d). The degree of capsular tissue is comparable to that formed when the blank polymer alone is present, suggesting no adverse reaction upon application of the combined therapeutic modality. The local tissue reaction typically consisting of thin

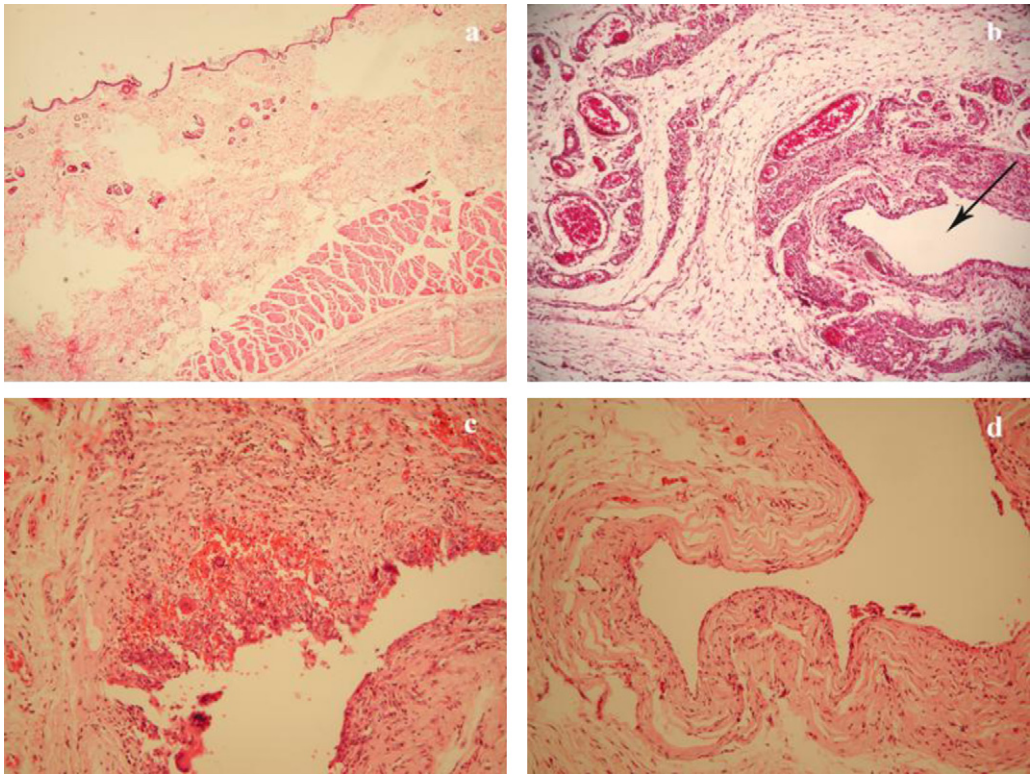

Fig. 9. Light micrographs of H&E-stained sections of subcutaneous tissue surrounding F4 implant after 0, 7, 28 and 42 days of implantation: (a) 0 day, (b) 7 days, (c) 28 days, and (d) 42 days. The implantation site for F4 implant is designated by a black arrow.

envelope capsule is interpreted as scarring chronic inflammation reaction. No evidence of granulomatous foreign-body or lymphoid cell aggregation indicates good compatibility of the implants.

4. Conclusion

In the present study, a series of PZQ-loaded implants based on PEG/PCL blends are successfully fabricated by HME. Drug particles are dispersed uniformly in the implants and keep stable during the fabrication process. Each component in the implants is mainly in its crystalline state. *In vitro* dissolution test demonstrates that by simply changing the composition of the PEG/PCL blends drug release behaviors from the implants can be effectively altered. PZQ release from all implants is primarily dominated by diffusion, and the cross-section morphologies of the implants after *in vitro* release are directly related to the PEG contents in the implants. For implants containing low contents of PEG (0–5%), the cross-sections primarily consist of discrete pores; while those of implants with high PEG contents (10–30%) consist of interconnected pores or channels. In comparison with the *in vitro* release, *in vivo* PZQ release becomes more moderate and follows zero-order model after an initial burst release. The implant can achieve sufficiently high drug concentrations in plasma over a period of at least 6 weeks and possesses excellent tissue compatibility.

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