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Research paper

A controlled release system of titanocene dichloride by electrospun fiber and its antitumor activity *in vitro*

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ABSTRACT

In order to improve both safety and efficacy of cancer chemotherapy of titanocene dichloride and overcome the shortcomings such as instability and short half-life in the human body, we report a controlled release system of titanocene dichloride by electrospun fiber and its *in vitro* antitumor activity against human lung tumor spca-1 cells. The system was developed by electrospinning. The release profiles of titanocene dichloride in PBS were researched by UV–Vis spectrophotometer. *In vitro* antitumor activities of the fibers were examined by MTT method. Titanocene dichloride was well incorporated in biodegradable poly(L-lactic acid) fibers. XRD results suggest that titanocene dichloride exists in the amorphous form in the fibers. The controlled release of titanocene dichloride can be gained for long time. MTT showed actual titanocene dichloride content 40, 80, 160 and 240 mg/L from the fibers mat, cell growth inhibition rates of 11.2%, 22.1%, 44.2% and 68.2% were achieved, respectively. The titanocene dichloride released has obvious inhibition effect against lung tumor cells. The system has an effect of controlled release of titanocene dichloride and may be used as an implantable anticancer drug in clinical applications in the future.

1. Introduction

The pharmaceutical industry has been successful in developing many new cytotoxic drugs, which have potential applications for cancer treatment, but cancer remains a serious threat to the human healthy and is a leading cause of death. Because of nonspecific uptake by normal, healthy, noncancerous tissues, current anticancer drug therapy leads to systemic side effects. Therefore, there have been numerous researches in order to develop more efficient release systems, decrease side effects and improve selective toxicities against cancer cells [1]. So far, many organic anticancer drugs have been investigated. However, the investigations on inorganic anticancer drugs are minor, such as cisplatin. Though these drugs have a side effect and some shortcomings such as instability and short half-life in the human body, research has shown that these drugs can show obvious antitumor activities [2,3]. Therefore, it is important to develop new systems to increase efficiency of inorganic anticancer drugs and decrease side effects. Among the inorganic anticancer drugs, there have been some reports on developing efficient systems of cisplatin, but only a few reports on developing systems of other inorganic anticancer drugs.

Titanocene dichloride is an organometallic compound and is widely used in organometallic and organic synthesis both as a reagent and as a catalyst. It is existed as a bright red solid, forming acicular crystals when crystallized from toluene. Titanocene dichloride is a kind of inorganic antitumor agent. Since Köpf first found that titanocene dichloride has obvious antitumor activity [4], it has been extensively researched [5,6]. It was reported that titanocene dichloride have antitumor activity against many tumor cells. Compared with cisplatin, it has better antitumor activity against colonic adenoma [2,7]. So far, titanocene dichloride has reached phase I clinical trials, with a maximum tolerable dose of 315 mg/m^2 per week. Unfortunately, there are two shortcomings in its application. One is that it has a low solubility; the other is that it has the short half-life in the human body. It was reported that the efficacy of titanocene dichloride in phase II clinical trials in patients with metastatic breast cancer and metastatic renal cell carcinoma was too low to be pursued [8,9]. So, it is very important to improve both safety and efficacy of cancer chemotherapy of titanocene dichloride.

To avoid the systemic toxicity associated with chemotherapeutic drugs and maintain their therapeutic concentrations in the local

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region of tumors, several ways have been researched. For example, controlled release of anticancer drug is a promising way [10–12]. However, to the best of our knowledge, there has not been reported on controlled release of titanocene dichloride to improve both safety and efficacy of cancer chemotherapy in the literature.

Research shows that a suitable controlled release system is important to improve both safety and efficacy of cancer chemotherapy. In the past several years, some drug delivery systems have been developed, such as liposomes [13], micelles, inorganic nanostructures [14], and nanoparticles [15]. One of the possible ways is incorporating the drugs into the polymer carriers [16,17]. With the development of the electrospinning technology, the use of the polymer electrospun fibers as drug carriers will be promising in the future biomedical applications, especially postoperative local chemotherapy, because the fibers have many advantages, such as reduced toxicity, improved therapeutic effect and convenient operation [18–24]. In addition, electrospinning is a relatively inexpensive manufacturing technique for submicron and nanometer diameter fibers. There have been some reports on the preparation of drug-loaded fibers by electrospinning. For example, Kenawy et al. first reported the release of 5% tetracycline hydrochloride from electrospun poly(ethylene-co-vinyl acetate), poly(lactic acid) and their blends [25]. Wang et al. report that electrospun microfibers and nanofibers for sustained delivery of paclitaxel to treat C6 Glioma *in vitro*. The results showed paclitaxel sustained release was achieved for more than 60 days [26]. Jing et al. developed implantable BCNU-loaded poly (ethylene glycol)-poly (L-lactic acid) diblock copolymer fibers for the controlled release of 1,3-bis (2-chloroethyl)-1-nitrosourea [27]. The results show the BCNU is continuously released in its active form from the PEG–PLLA fiber mats and the shortcomings of BCNU such as instability in ambient atmosphere and short half-life in the human body can be overcome to a certain extent by incorporating it into the electrospun fibers.

In this full paper, we first report a controlled release system of titanocene dichloride by electrospun fiber and its *in vitro* antitumor activity against human lung tumor spca-1 cells. Titanocene dichloride can be released in its active form from the fibers. The titanocene dichloride released from the fibers has an obvious inhibition activity against tumor spc-a-1 cells. The results suggest that the release system has an effect of controlled release of titanocene dichloride and titanocene-dichloride-loaded PLLA fibers may be used as an implantable anticancer drug in clinical applications in the future.

2. Materials and methods

2.1. Materials

PLLA (molecular weight 100,000) was purchased from Shandong Jianbao biomaterials Ltd. (China). Titanocene dichloride was donated by Yueyang Sanshou Ltd. (China). All other chemical solvents were obtained from Chemical Reagent Co. Ltd. (Shanghai, China) and used without further purification. RPMI 1640 and Newborn Calf serum were purchased from Shanghai Shichen Reagent Co. Ltd. (Shanghai, China). Proteinase K was purchased from Sigma. Human lung tumor spc-a-1 cell was newly purchased from the Shanghai cell center (Chinese Academy of Sciences).

2.2. Methods

2.2.1. Fabrication of the products

In our experiment, 5 g PLLA was dissolved in 50 mL dichloromethane to form a solution by using a bath sonicator (KQ-100, China). Then a predetermined amount of titanocene dichloride powders was dispersed in dichloromethane. The PLLA solution was added to the titanocene dichloride solution by continuous stirring. A high voltage DC generator was purchased from Beijing Machinery & Electricity Institute. A syringe pump (model WZ-50C2) was purchased from Zhejiang University Medical Instrument Co., Ltd.

In a typical procedure of electrospinning, first, the polymer solution was transferred to a glass syringe and feed. Then, the syringe pump was used to deliver the solution through Teflon tube connecting the syringe with the inner capillaries (needles). A high voltage DC generator was used to produce a 12–22 kV voltage to spin solution through the inner needle. A collector of aluminum foil was used to collect the random fibers. The distance from the spinneret to the collector was fixed at 15 cm. All the experiments were performed at room temperature. Finally, the fibers were taken out and dried under vacuum at room temperature for 48 h. The blank fibers without titanocene dichloride incorporation were fabricated by the same method.

Dichloromethane residue was determined by Capillary Gas Chromatography. The HP-FFAP (PEG) capillary column (50 m $\times$ 0.33 mm $\times$ 0.32 μm) and FID detector were adopted. Detector temperature is 220 °C, and the column temperature is 180 °C. We have used a calibration curve of dichloromethane to determine the residue. Dichloromethane was dissolved in the dimethyl sulphoxide. The fibers (1.0000 g) were dissolved in the dimethyl sulphoxide to determine the dichloromethane residue.

2.2.2. Characterization of the products

A scanning electron microscopy (SEM) equipped with an energy-dispersive spectroscopy (EDS) accessory was used to observe both the morphology of collected fibers and Ti element in the fiber. The samples for SEM observation were sputtered coated with gold. The fiber diameter of the electrospun fibers was measured with software Image J. The average fiber diameter and its distribution were determined from the random fibers upon a typical SEM image. The titanocene dichloride distribution on the surface of PLLA fibers was investigated by an EDS. The structure of the electrospun fibers was examined by X-ray diffraction (XRD) diffractometer. The XRD patterns were determined with an X-ray diffractometer with Cu Kα radiation (λ = 0.15405 nm, 40 kV, 100 mA) over the 2θ range of 10–70° with the scanning rate of 10°/min. The DSC data was obtained by a DSC STA 449C Jupiter thermal analysis instrument. About 10 mg sample in an aluminum pan was heated from 40 to 500 °C at a heating rate of 10 °C/min.

2.2.3. Determination of the loaded drug

The titanocene-dichloride-loaded PLLA fibers were dissolved in dichloromethane. The concentration of titanocene dichloride in the fibers was measured by Agilent UV–Vis spectrophotometer at 390 nm.

2.2.4. Drug release test in vitro

We determined the amount of titanocene dichloride and got the release profiles of titanocene dichloride in PBS by UV–Vis spectrophotometer at 243 nm. A fiber mat was used for the release studies. The titanocene-dichloride-loaded PLLA fiber mat (about 100 mg each) was statically incubated in 30 ml phosphate buffer solution (pH 7.4) at 37.0 °C. At preset interval, 1 mL of the incubated solution was taken out and measured by UV–Vis spectrophotometer by using the incubated solution of blank fibers as control. The accumulative release of titanocene dichloride from the fiber was calculated as the function of incubation time. The drug-loaded fibers were evenly divided into three parts and were incubated in the PBS without proteinase K, in the PBS with 10 mg/L proteinase K and in the PBS with 50 mg/L proteinase K, respectively. The experiments were performed for three times on the same condition for each set. The release profile presents n = 3 for each fiber batch.

2.2.5. Antitumor activities in vitro

In vitro antitumor activities of the fibers were examined by MTT method. Human lung tumor cells (spc-a-1 cell line) were chosen as the target tumor cells. The tumor cells cultured in RPMI 1640 containing newborn calf serum were adjusted to 5×10^4 cells/mL; 200 μL aliquots of the cell suspension were added into each well of a 96-well plate and incubated in humidified atmosphere containing 5% CO_2 at 37.0 °C for 24 h. And then, titanocene-dichloride-loaded PLLA fiber mat were added to the tumor-cell-cultured well and incubated for another 24 h. Titanocene dichloride contents of the fiber mat were 4, 8 and 16% with respect to the polymer weight used. Actual titanocene dichloride contents were 40, 80,160 and 240 mg/L. Then, the 20 μL MTT solution (5 mg/mL) was added to each well and kept for 4 h of incubation. Finally, the solution in the wells was deserted completely and 150 μl DMSO was added to each of the wells to dissolve the residue. The optical densities of DMSO solutions were determined by a microplate reader at 490 nm and the cell inhibitions were calculated. The optical density values in one group were average. The relative cell viability rate was calculated by dividing the optical density value of the test group by that of the control group.

3. Results and discussion

3.1. Characterization of the products

In this study, we select PLLA as a carrier because it has a number of important characteristics such as biocompatible and biodegradable characteristic [28,29]. Titanocene dichloride was easily dissolved in PLLA/dichloromethane solution. The mixture solution was stable and homogeneous. SEM images of the typical samples are shown in Fig. 1a. Fig. 1b shows the EDS image. The average diameters measured by image software Image J were 1500 nm. The fiber morphology shows the surface is smooth and no titanocene dichloride crystals are detected. The fibers look uniform. Moreover, the EDS result of the Ti element shows that the titanocene dichloride can be detected in the fibers. These results show that titanocene dichloride is finely incorporated into the fibers.

To demonstrate the physical state of titanocene dichloride in the fibers, titanocene-dichloride-loaded PLLA fibers, titanocene dichloride powder and PLLA fibers were characterized by XRD. Fig. 2 shows XRD patterns of the titanocene dichloride powder, PLLA fibers and titanocene-dichloride-loaded PLLA fibers. As shown in Fig. 3A and B, titanocene dichloride is crystalline, with characteristic peaks at $2\theta = 13.9°$, 15.7°, 19.8°, 32.1°, respectively, while PLLA fibers are amorphous. Fig. 3C shows the crystalline titanocene dichloride is not detected in the titanocene-dichloride-loaded PLLA fibers. This suggests that titanocene dichloride exists in the amorphous form, probably as amorphous molecular aggregates or a solid solution in the fibers.

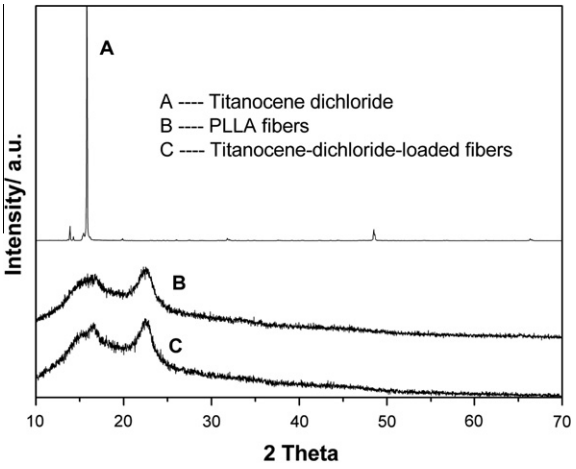

Fig. 2. XRD patterns of the samples: (A) titanocene dichloride powder, (B) PLLA fibers and (C) titanocene-dichloride-loaded PLLA fibers.

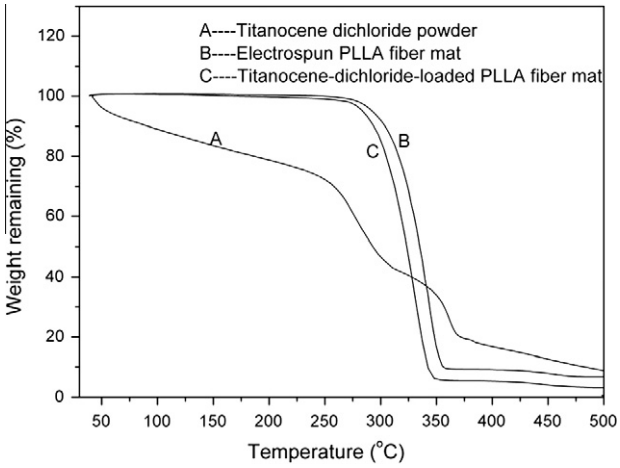

Fig. 3. Thermogravimetric analytical thermograms of the products: (A) titanocene dichloride powder, (B) electrospun PLLA fibers and (C) titanocene-dichloride-loaded PLLA fiber mat (heated from 40 to 500 °C at a heating rate of 10 °C/min).

Fig. 3 shows TGA thermograms for the titanocene dichloride powder (Fig. 3A), PLLA fibers (Fig. 3B) and titanocene-dichloride-loaded PLLA fibers (Fig. 3C). Fig. 3A exhibits four steps in the mass loss of the titanocene dichloride powder, with the first covering the temperature range of 60–255 °C, the second covering the temperature range of 255–308 °C, the third covering the temperature range of 308–351 °C and the fourth covering the temperature

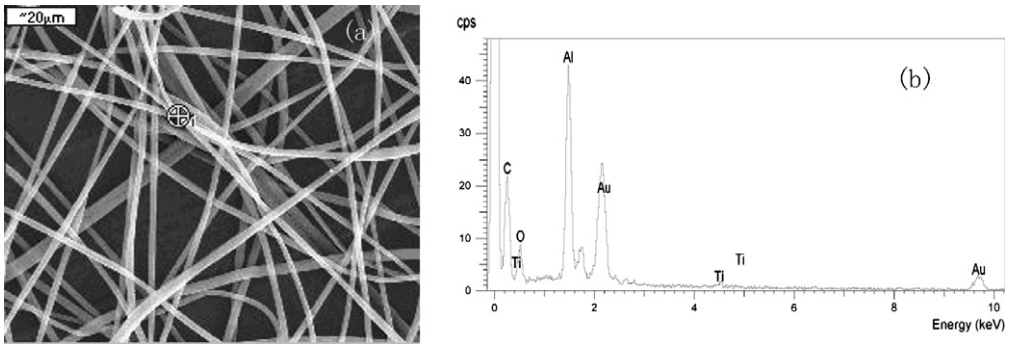

Fig. 1. SEM micrograph (a) and EDS micrograph (b).

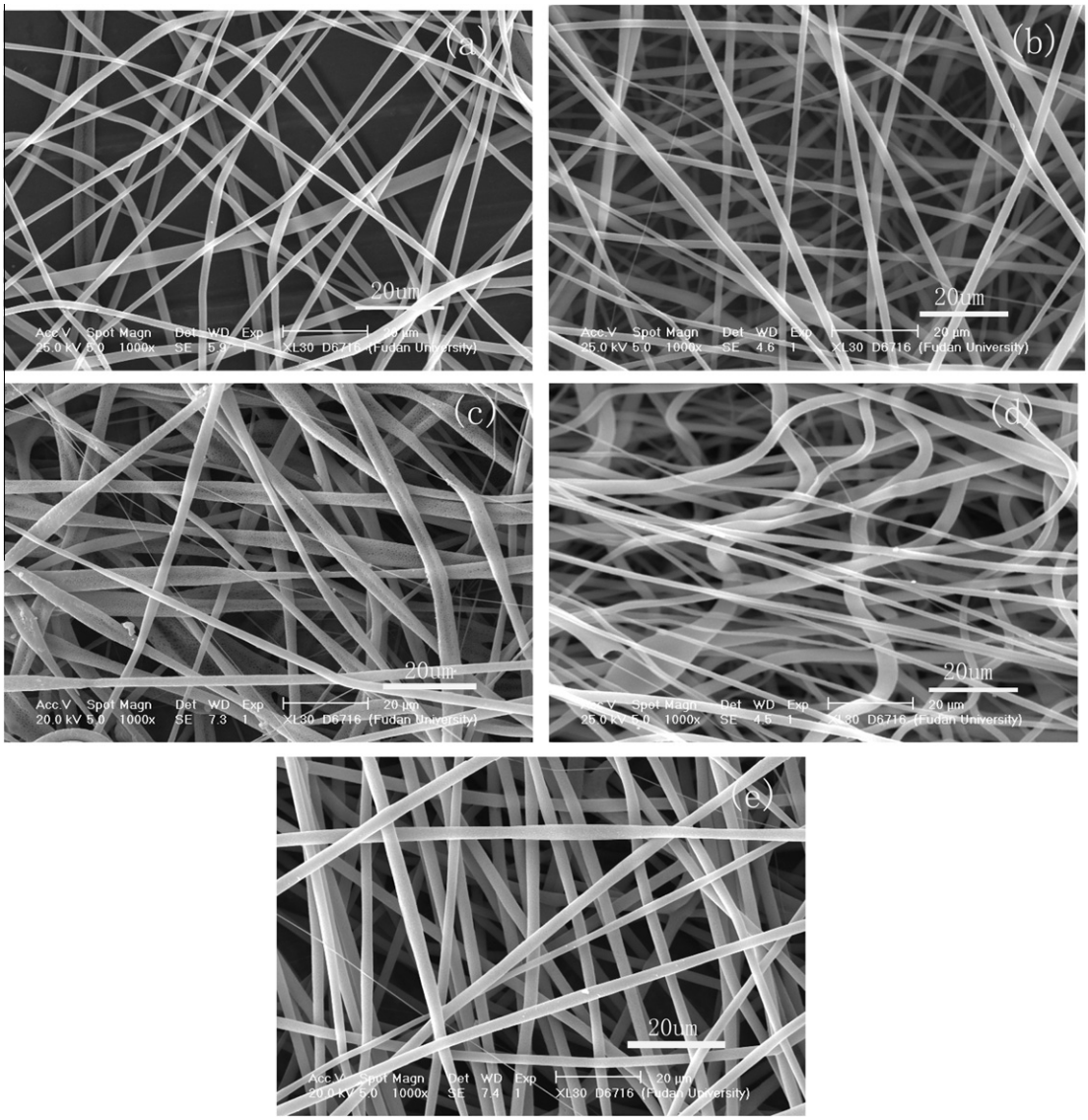

Fig. 4. SEM micrographs of the products: (a) F1 fiber, (b) F2 fiber, (c) F3 fiber, (d) F4 fiber, (e) PLLA fiber.

Table 1
Processing parameters on the fibers fabricated by electrospinning and characterization (voltage: 16 kV, flow rate: 6 mL/h).

Sample	PLLA/titanocene dichloride (w/w)	Size (μm) ± SD	Drug loading (%)	Tensile stress (MPa) and strain (%)
F1	50 mg/2 mg	1.52 ± 0.65	4.0	3.55, 48.80
F2	50 mg/3 mg	1.75 ± 0.72	5.4	3.03, 34.66
F3	50 mg/4 mg	2.26 ± 1.16	7.8	2.21, 36.10
F4	50 mg/5 mg	2.52 ± 1.75	9.7	2.07, 32.45

range of 351–380 °C. Fig. 3B shows the mass loss of the PLLA fibers over the temperature range of 300–360 °C. Fig. 3C exhibits the mass loss of titanocene-dichloride-loaded PLLA fibers over the temperature range of 285–345 °C. For Fig. 3A, firstly, the temperature range of 60–255 °C is because of the decomposition of the two chlorines. Secondly, the temperature range of 255–308 °C is because of the decomposition of the first cyclopentadienyl group. The temperature range of 308–351 °C is because of the decomposition of the second cyclopentadienyl group. Fig. 3C exhibits the mass loss of titanocene-dichloride-loaded PLLA fibers (drug loading 5.4%). Because the content of the loaded titanocene dichloride is low, the analytical thermograms of Fig. 3C and B are similar and only slight difference in the temperature range. These results confirm the presence of the titanocene dichloride within the PLLA fibers.

Dichloromethane residue was determined by Capillary Gas Chromatography. The results show dichloromethane was not detected. Therefore, we believe that dichloromethane residue meet the requirements of European Pharmacopoeia.

3.2. Fabrication conditions of the products

The effects of key processing parameters on the fibers fabricated by electrospinning such as polymer concentration, titanocene

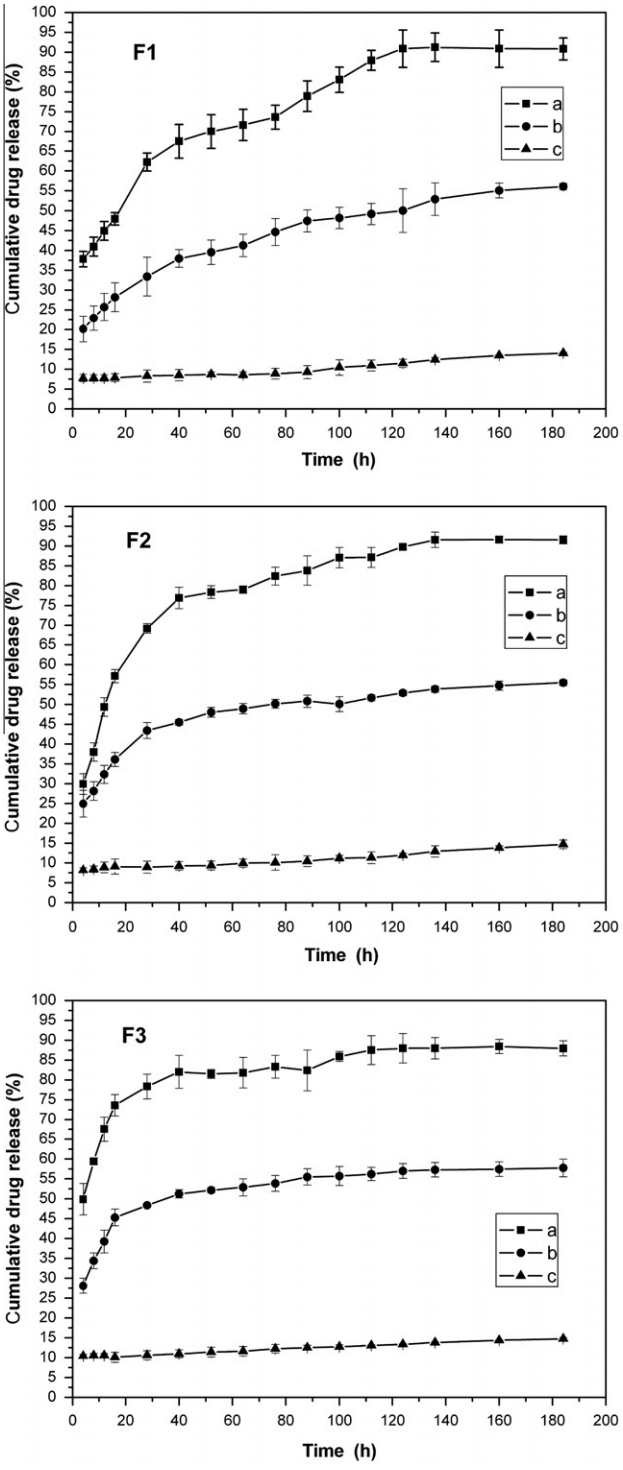

Fig. 5. *In vitro* release profiles of titanocene dichloride from the fiber F1 (F1), fiber F2 (F2) and fiber F3 (F3). Each data point represents the average of n = 3 samples, error bars represent standard deviations. (curve a: in the PBS with 50 mg/L proteinase K, curve b: in the PBS with 10 mg/L proteinase K, curve c: in the PBS without proteinase K). Release condition: pH 7.4 phosphate buffer solution, 37.0 °C.

dichloride concentration, electrospinning voltage, polymer solution flow rate, etc. were investigated. The detailed results were shown in Supporting information. The diameters from about 1.5 μm to 2.0 μm of the fibers could be fine tuned by adjusting the processing parameters. Fig. 4 shows the SEM images of the products fabricated on the different processing parameters. The

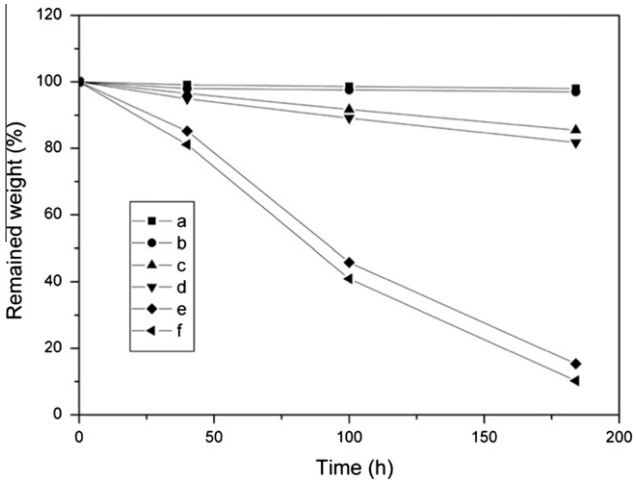

Fig. 6. Remained weight of the blank PLLA fiber and F1 sample in PBS, PBS with 10 mg/L proteinase K and PBS with 50 mg/L proteinase K (weight was the average of triplicate samples) (curve a: blank PLLA fiber in PBS, curve b: F1 fiber in PBS, curve c: blank PLLA fiber in PBS with 10 mg/L proteinase K, curve d: F1 fiber in PBS with 10 mg/L proteinase K, curve e: blank PLLA fiber in PBS with 50 mg/L proteinase K, curve f: F1 fiber in PBS with 50 mg/L proteinase K.

diameters of F1, F2, F3 and F4 fiber is about 1.5, 1.7, 2.2 and 2.5 μm, respectively. Titanocene dichloride content in the F1, F2, F3 and F4 fibers is 4.0%, 5.4%, 7.8% and 9.7%, respectively. The fiber morphologies show the titanocene dichloride can be loaded in the fibers. The characterization of typical samples obtained in the present study is shown in Table 1. In the experiment we found, when the amount of the titanocene dichloride was more than 15% with respect to PLLA, the fibers contain a few bead-shaped products. When the amount was more than 20%, the products mainly were bead-shaped products instead of fibers. We regard the fibers as the products. Therefore, we think that 15% is the maximum amount of drug that can be loaded in the nanofiber mat.

Tensile stress and strain of the F1, F2, F3 and F4 fiber were measured by the economic tensile strength testing machine (Instron). The data were shown in Table 1.Tensile stress and strain of the blank PLLA fiber is 3.69 MPa and 47.95%. The results show a trend that the more titanocene dichloride in the fibers, lower tensile stress. However, tensile stress and strain of the F1, F2, F3 and F4 fiber were adequate in the potentially application as an implantable release system.

3.3. Determination of titanocene dichloride concentration in the PBS

The titanocene dichloride concentration in the PBS solution was determined by UV–Vis spectrophotometer at 243 nm. The absorption of titanocene dichloride was proportional to the concentration of titanocene dichloride within the range of 3–24 mg/L with a correlation coefficient of 0.9989. The linear regression equation was $Y = -0.05921 + 0.03643X$ (Y is the absorption and X is the concentration of titanocene dichloride). The method can determine the content of the titanocene dichloride in the PBS. Therefore, we measured the amount of the titanocene dichloride and got the release profiles of titanocene dichloride in PBS by UV–Vis spectrophotometer. In protease solution (10 mg/L), the linear regression equation was $Y = -0.0340 + 0.03124X$ (Y is the absorption and X is the concentration of titanocene dichloride). The correlation coefficient is 0.9980. In protease solution (50 mg/L), the linear regression equation was $Y = 0.00221 + 0.02990X$ (Y is the absorption and X is the concentration of titanocene dichloride). The correlation coefficient is 0.9991.

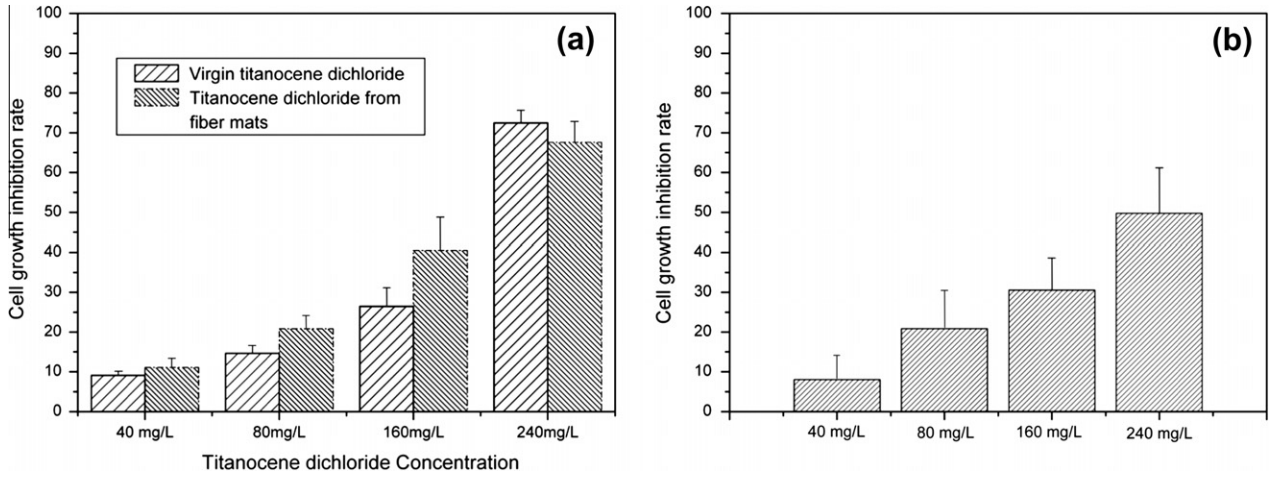

Fig. 7. Antitumor activities of the fibers mat to human lung tumor spc-a-1 cells PLLA /titanocene dichloride fiber mats were directly added to the tumor-cell-cultured well and incubated for 24 h (a) and 48 h (b).

3.4. Drug release in vitro

Fig. 5F1–F3 showed the release profiles of titanocene dichloride from the fiber F1, fiber F2 and fiber F3, respectively. The curve a showed the release profiles of titanocene dichloride from fiber in the PBS with 50 mg/L proteinase K. The curve b showed the release profiles of titanocene dichloride in the PBS with 10 mg/L proteinase K. The curve c showed the release profiles of titanocene dichloride in the PBS without proteinase K. Fig. 5F1 showed a trend that the release rate of titanocene dichloride from fiber F1with 10 mg/L proteinase K is faster than that without proteinase K, the release rate with 50 mg/L proteinase K is faster than that with 10 mg/L proteinase K. From Fig. 5F2 and F3, the same trend can be shown. Proteinase K can increase the release of titanocene dichloride from fiber, because it can speed the degradation of the PLLA. Proteinase K was selected as enzyme for release studies. There are two reasons for this. First, proteinase K may enhance the degradation of the polymer to quicken the release of the titanocene dichloride, and it is convenient to study. Second, it simulates the surrounding including enzyme by adding proteinase K.

Initial fast release of titanocene dichloride from the fiber was lower in the experiments without proteinase K and with 10 mg/L proteinase K. The results indicate titanocene dichloride finely incorporated into the fibers. From the curves a and b (Fig. 5F1), we found that the release rate is higher before 40 h and then it is lower. The same trend can be found in Fig. 5F2. However, from the curve a and b (Fig. 5F3), we found that the release rate is higher before 30 h, and then it has lower release rate. We primarily believe it is because the release rate was affected by not only mean diameter of the fibers but also the content of titanocene dichloride.

We select PLLA as the delivery vehicle. First, it has a number of important characteristics such as biocompatible and biodegradable characteristic. Second, PLLA is a relatively hydrophobic polymer because of the methyl group in its structure and therefore inherently slower biodegrading. By the electrospinning technology, we prepared the fibers which have higher drug loading. About 15% is the maximum amount of drug. We want to combine the two characteristics of the PLLA and electrospinning technology and obtain the delivery system for long time to improve the performance of the titanocene dichloride. Fig. 5 indicates that the controlled release of titanocene dichloride from the fiber F1, F2 and F3 can be gained for long time.

Titanocene dichloride has extensively antitumor activities [5]. Unfortunately, the efficacy of titanocene dichloride in phase I clinical trials in patients with metastatic breast cancer and metastatic renal cell carcinoma was too low to be pursued. The disappointing outcome possibly stems from problems in the stable formulation of the drug for clinical use and having a short half-life in the human body [30,31]. Therefore, this method is very meaningful to control the release of titanocene dichloride and improve both safety and efficacy of cancer chemotherapy and overcome the shortcomings such as instability and short half-life in the human body.

It was reported that the release profile of drug from PEG-PLLA matrix is mainly controlled by not only diffusion of the drug but also degradation of the matrix [17,32]. We researched the degradation of the nanofiber matrix in the presence and absence of proteinase K, respectively. We tested the weight loss of the blank PLLA (MW = 100,000) electrospun fibers and the electrospun fibers (F1 sample) in the PBS, the PBS with 10 mg/L proteinase K and with 50 mg/L proteinase K within 184 h. The results were shown in Fig. 6. The results show that 3.9% of the weight loss of the F1 sample was observed in PBS within 184 h. For the blank PLLA electrospun fibers, 3.0% of the weight loss was observed in PBS within 184 h. Therefore, we believe that 3.9% of the weight loss of the F1 sample mainly is due to the degradation of the nanofiber. These results demonstrate that titanocene dichloride release mainly depends on the degradation of the nanofiber.

Because PLLA has the good biocompatible and biodegradable characteristic in the human body, titanocene dichloride-loaded PLLA fibers by electrospinning may be degraded in the human body. So developing the controlled release system of titanocene dichloride from electrospun fibers is of great importance. The fibers may be used as an implantable device for malignant tumor and improve both safety and efficacy of cancer chemotherapy.

3.5. Antitumor activity in vitro

Since the early 1980s, titanocene dichloride has been extensively researched. It was reported that it has shown very effective antitumor activity against lung carcinoma, Ehrlich ascites tumors, colon B adenocarcinoma and B16 melanoma [9,33–35]. In this study, we researched antitumor activity of the fibers against human lung tumor spc-a-1 cells. MTT assay was used to test the antitumor activity *in vitro*. We conducted this with fibers with different loading and used titanocene dichloride solution as a positive control. The fiber mats were directly added to the tumor-cell-cultured well and incubated for 24 h. The results are shown in Fig. 7a. In the cases of actual titanocene dichloride content 40, 80, 160 and 240 mg/L from the fibers mat, cell growth inhibition rates of 11.2%, 22.1%, 44.2% and 68.2% were achieved, respectively. For

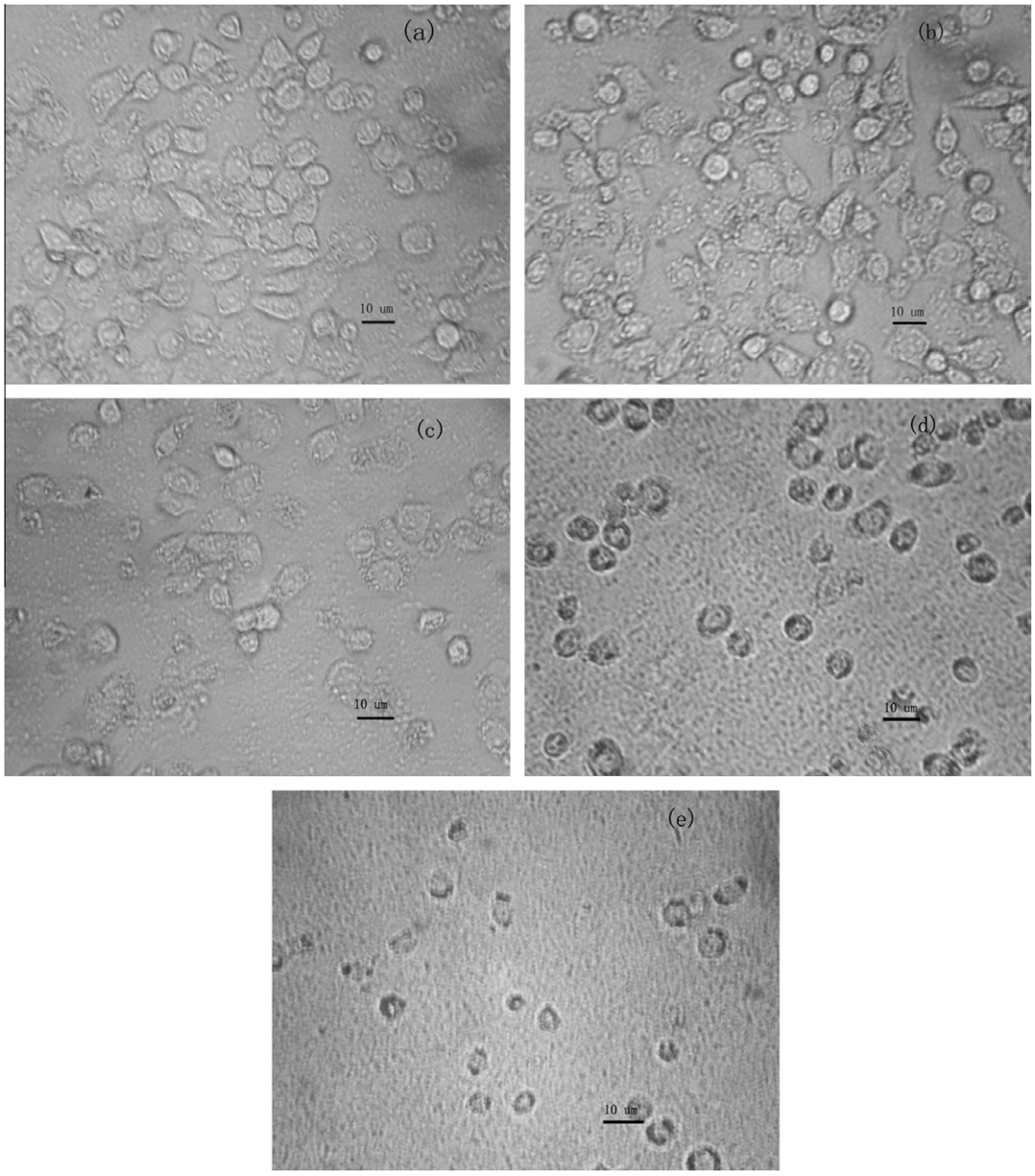

Fig. 8. Cell morphologies of the human lung tumor spc-a-1 cells treated (a) control (non-treated), (b) blank PLLA fibers mats, (c) actual titanocene dichloride content: 40 mg/L, (d) actual titanocene dichloride content 80 mg/L (e) actual titanocene dichloride content: 160 mg/L.

the virgin titanocene dichloride content of 40, 80, 160 and 240 mg/L, cell growth inhibition rates of 10.0%, 17.0%, 29.2% and 73.1% were achieved, respectively. That is to say, the same content of titanocene dichloride from virgin titanocene dichloride and titanocene dichloride-PLLA fibers has almost equal antitumor activity *in vitro*. IC50 value (concentration of titanocene dichloride able to inhibit the growth of spc-a-1 cells to 50% of the control) of the titanocene alone and after loading in the fibers have been determined. The results show that IC50 value of the titanocene dichloride alone and after loading in the fibers were 185.75 mg/L and 165.70 mg/L. The results show the titanocene dichloride released from the titanocene dichloride-PLLA fibers still have same inhibition effect. We also tested the antitumor activity of the fibers for 48 h. The results are shown in Fig. 7b. In the cases of actual titanocene dichloride content 40, 80, 160 and 240 mg/L from the fibers mat, cell growth inhibition rates of 8.3%, 21.7%, 30.3% and 50.6% were achieved, respectively.

Fig. 8 shows the cell morphologies of the human lung tumor spca-1 cells treated by the fibers with different loading titanocene dichloride. As shown in Fig. 8, the blank PLLA fibers did not show obvious antitumor activity, compared with the control. Fig. 8c–e shows the cell morphologies of the cells directly treated by the fibers mat. Actual titanocene dichloride content was 40, 80 and 160 mg/L, respectively. The cell numbers decrease obviously during the test and the cell morphologies changed obviously. More titanocene dichloride included in the fibers shows stronger cell growth inhibition.

From these results, we believe that the titanocene dichloride-PLLA fibers are a sustained delivery system and titanocene dichloride can continuously be released in an active form from the system. Therefore, the shortcomings of titanocene dichloride such as instability and short half-life in the human body can be overcome in a certain degree. Incorporating titanocene dichloride into the fibers by electrospinning method is an ideal technique for improving

the performance of the titanocene dichloride. For lung cancer, surgery is the primary treatment for patients. But recurrence of lung cancer is very common. It is not very hard to remove the entire tumor [36,37]. We think these nanofibers can be used as an implantable drug delivery system for lung cancer after the surgery. This system can be used to prevent the recurrence of lung cancer after the surgery.

4. Conclusion

In this study, we first report a method to control the release of an organometallic anticancer drug to improve both safety and efficacy of cancer chemotherapy and overcome the shortcomings. The controlled release system of titanocene dichloride by electrospun fibers was developed. Titanocene dichloride is finely incorporated into the fibers. Controlled release of titanocene dichloride can be gained for long time. The titanocene dichloride released from the fibers has obvious inhibition effect against human lung tumor cells. The results show the PLLA/titanocene dichloride composite fiber is an ideal controlled release system. The long-term delivery of titanocene dichloride may be useful in the development of new implantable polymeric devices for malignant tumor. This research will also be helpful for applications of the other inorganic anticancer drug. Studies on the release of titanocene dichloride in vivo and its anticancer activities will be performed in the future.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2010.09.005.

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