Review

Preparation of core-shell biodegradable microfibers for long-term drug delivery

Hui-Hua Huang,[1,2] Chuang-Long He,[2,3] Hong-Sheng Wang,[3] Xiu-Mei Mo[1,2,3]
[1]*College of Material Science and Engineering, Donghua University, Shanghai 201620, China*
[2]*State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Donghua University, Shanghai 201620, China*
[3]*Biomaterials and Tissue Engineering Laboratory, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China*

Received 1 April 2009; accepted 7 April 2009
Published online 1 July 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.32543

Abstract: A coaxial electrospun technique to fabricate core-shell microfibers (MFs) for drug delivery application is described. In one-step, Paclitaxel (PTX)-loaded poly(L-lactic acid-*co*-ε-caprolactone) (75:25) (P(LLA-CL)(core/shell)) was electrospun into MFs using 2,2,2-trifluoroethanol as the solvent. The physical and chemical properties of electrospun fibers were characterized by various techniques, such as scanning electron microscopy, transmission electron microscopy, X-ray diffractometry, and Fourier-transform infrared. The fiber diameter depended on both the polymer concentration and the flow ratio of PTX to P(LLA-CL). The encapsulation efficiency and *in vitro* release profile were measured using high performance liquid chromatography methods. PTX released from the MFs in a short burst over 24 h followed by very slow release over the following 60 days. In addition, the cytotoxicity of PTX-loaded P(LLA-CL) MFs was evaluated using 3-[4,5-dimehyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide assay on HeLa cell lines. These results indicate that PTX could be released from P(LLA-CL) fibers in a steady manner and effectively inhibit the activity of HeLa cells. J Biomed Mater Res 90A: 1243–1251, 2009

Key words: coaxial electrospinning; P(LLA-CL); paclitaxel; drug delivery; cytotoxicity

INTRODUCTION

Paclitaxel (PTX), a natural taxane isolated from the Pacific yew tree, Taxus brevifolia, is a potent antiproliferative agents for treatment of a wide range of cancers, such as ovarian, breast cancers, and non-small cell lung cancer.[1] PTX is currently being marketed as Taxol, (highly hydrophobic, with water solubility $\leq$0.5 mg/L),[2] in which PTX is dissolved in a mixture of 50% Cremophor Emulsifier (EL) and 50% dehydrated alcohol and given by intravenous (i.v.) infusion for clinical applications. Cremophor EL may cause serious side effects including severe hypersensitivity reactions, nephrotoxicity, neurotoxicity, and cardiotoxicity.[2–4] Over the past few years, PTX success is largely due to its unique action mechanism on microtubules, leading to the death of the cell by disrupting the normal tubule dynamics required for cell division and vital interphase processes.[2,3,5] Various approaches have been used for development of formulations of PTX to avoid using Cremophor EL, such as microspheres/nanospheres, liposomes, emulsions, water-soluble prodrugs, cyclodextrin complexes, polymeric micelles, polymeric paste, and microfibers/nanofibers. Also, various fabrication methods have been investigated to deliver PTX, for example, freeze-drying,[6] electrospinning,[7] solvent evaporation method,[8] self-assembly,[9] spray drying,[10] electrohydrodynamic atomization,[11] etc.

Recently, an increasing amount of attention has been paid to the use of the electrospinning technique to fabricate biodegradable polymers as drug delivery

Correspondence to: X.-M. Mo; e-mail: xmm@dhu.edu.cn
Contract grant sponsor: National Science Foundation; contract grant number: 30570503
Contract grant sponsor: National High Technology Research and Developed Program; contract grant number: 2008AA03Z305
Contract grant sponsor: Science and Technology Commission of Shanghai Municipality Program; contract grant numbers: 08520704600, 0852nm03400
Contract grant sponsor: Biomedical Textile Materials Science and Technology; contract grant number: B07024

systems. Electrospinning is a simple and versatile technique for producing microfibers (MFs) with diameters ranging from a few nanometers to microns. Polymer ultrafine fibers have some advantages of three dimensional porous structures and large surface area.[12] For drug delivery application, polymer ultrafine fibers can offer rapid, immediate, or delayed manner drug release in the light of the condition of the drugs incorporated into the polymer carrier. Many studies have reported that representative biodegradable polymers including synthetic ones such as poly(L-lactic acid) (PLLA),[7] copolymer of ε-caprolactone and ethyl ethylene phosphate (PCLEEP),[13] poly(lactic-*co*-glycolic acid) (PLGA),[14] poly(ε-caprolactone),[12] poly(ε-caprolactone)/poly(ethylene glycol) (PCL/PEG),[15] poly(ethylene glycol)-poly(L-lactic acid) (PEG-PLLA),[16] (50:50) poly(L-lactic acid-*co*-ε-caprolactone) [P(LLA-CL)],[17] or natural ones such as collagen,[18] silk fibroin,[19] and chitosan[20] have been electrospun into nanofibers for diversity drug delivery systems. These studies suggested that ultrafine fibers made from native polymers were better than those from synthetic polymers in the interaction between cells and carrier materials, but the synthetic polymers can provide more favorable mechanical properties than the native polymers. The drug release characteristic depends on how well the drug is encapsulated inside a carrier, due to rapid evaporation of the solvent in the mixture during electrospinning. The high ionic strength of spinning dopes make the drug particles in the polymer fibers easy to locate on the fiber surface.[21] Therefore, entrapment and sustained release of drug particles by conventional electrospinning techniques remains challenging.[22] An advanced coaxial electrospun technique has been recently described.[12,21] Compared with conventional electrospinning, coaxial electrospinning is essentially a modification or extension of the former with a major difference in the configuration of spinneret. As drug carriers, core-shell structure fibers electrospun from coaxial electrospinning can potentially provide a better therapeutic effect, reduced toxicity, and sustained drug release. For example, the core-shell structured fibers fabricated by coaxial electrospinning of PCL as the shell and Bovine Serum Albumin (BSA)-loaded PEG as the core can release BSA sustainably for more than 30 days.[12]

P(LLA-CL) is one of the most widely studied biodegradable polymers. P(LLA-CL) has been employed in pharmaceutical and biomedical fields owing to its unique properties, such as biocompatibility, nontoxicity, and biodegradability. These characteristics make P(LLA-CL) an excellent candidate for various biomedical applications, such as drug delivery, gene delivery, and tissue engineering.[17]

In the present study, the main objectives were to encapsulate PTX into core-shell fibers as topical drug delivery devices to treat HeLa cells and to determine the effect of different inner feeding rates on drug release rate *in vitro*.

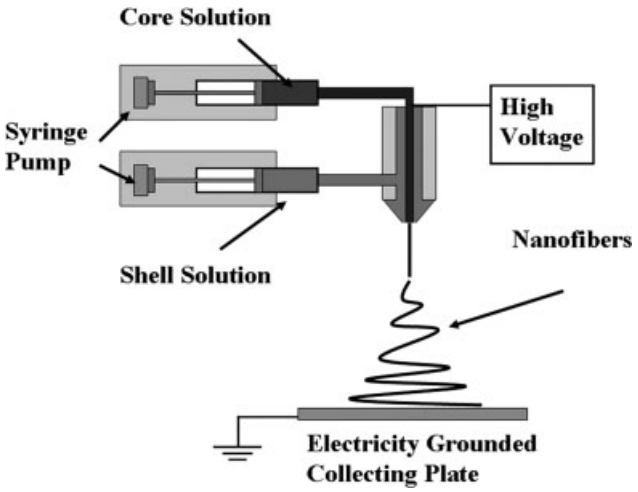

Figure 1. Schematic representation of the coaxial electrospinning setup.

MATERIALS AND METHODS

Materials

The block copolymer of P(LLA-CL) (M_w = 1.3–1.4 × 10^5, Japan), which is composed of 75% L-lactide, was used. PTX was purchased from Parling Pharma Tech Co., Ltd. (Shanghai, China). 2,2,2-Trifluoroethanol (TFE) was obtained from Shanghai Finechenm Co., Ltd (Shanghai, China). Tween-80 and sodium azide were purchased from Sigma. Acetonitrile [high performance liquid chromatography (HPLC) grade] was supplied by Merck. HeLa cells were donated by Chinese Academy of Sciences. Unless otherwise noted, all culture media and reagents were purchased from Gibco Life Technologies Co., USA. The polymers and solvents for electrospinning were used as received without further purification.

Coaxial electrospinning

The two liquids were independently fed through two needles to facilitate the formation of a stable jet. A TFE solution of PTX (19 mg/mL) was used as the core fluid; a solution of P(LLA-CL) in TFE with a concentration of 80 mg/mL was prepared as the shell polymer fluid for MFs via the coaxial electrospinning. The basic experimental setup was schematically shown in Figure 1. The exit orifice diameters of outer and inner capillaries are 1.2 and 0.5 mm, respectively. The inner layer and outer layer flow rates were adjusted by two separate syringe pumps. The inner feed rate ranges from 0.4 to 0.8 mL/h, whereas the outer feed rate was constant at 1.2 mL/h. The voltage of 12.5 kV was applied to the needle using a high-voltage power supplier (BGG6-358, BMEI CO. LTD., China). A

grounded collection plate of aluminum foil was located at a fixed distance 19 cm from the needle tip. Previous experimental results showed that the very low ($\leq$20 mg/mL) concentration of P(LLA-CL) solution was hard to generate the core-shell structured fiber and to be electrospun into any fiber form owing to the lack of an adequate viscosity for jet formation.[21]

Scanning electron microscopy

The surface morphology of the MFs was observed on a JEOL JSM-5600LV scanning electron microscopy (SEM) at an acceleration voltage of 10–15 kV. Samples for SEM were dried under vacuum, mounted on metal stubs, and sputter-coated with gold. The average diameters and size distribution of the fibers were analyzed with the Image J_1.34 (National Institutes of Health, USA). In each case, average fiber diameter and diameter distribution were determined from about 100 measurements of the random fibers in a typical SEM image.

Transmission electron microscopy

The core-shell structure of the fibers was observed using a transmission electron microscopy (TEM) operated at 100 kV. The samples for TEM were prepared by directly depositing the as-spun MFs onto TEM carbon-coated copper grids. The samples were kept in a vacuum oven for drying at room temperature before the TEM imaging.

X-ray diffractometry

The X-ray diffractometry (XRD) for pure PTX, P(LLA-CL) as-received, and PTX-loaded P(LLA-CL) MFs were obtained with a D/Max-2550PC diffractometer (Rigaku, Japan). The measurements were under Cu Kα1, 40 kV and 300 mA as X-ray source. The diffraction patterns were collected with 2θ ranging from 5° to 60°.

Fourier-transform infrared

PTX powder, P(LLA-CL) as-received, and MF were prepared for the Fourier-transform infrared (FTIR) test on AVATAR 380 FTIR instrument (Thermo Electron, Waltham, MA).

In vitro release studies of PTX and encapsulation efficiency

PTX release from MFs was carried out over a period of days *in vitro*. Samples were put in capped glass tubes containing 10 mL of phosphate buffered saline (PBS, pH 7.2) containing 0.05% (w/v) Tween-80 to enhance the solubility of PTX and 0.1 mg/mL of sodium azide. The tube was incubated in a shaker bath at 37°C, 90 rpm. Three milliliters of sample mixture were extracted at intervals from each test tube. At the same time, PBS solution was placed back as same as the sample in tube, and all the mixtures were incubated in the shaker bath again before the next set of sample mixtures was extracted. PTX in the release medium was first extracted with 1 mL of dichloromethane (DCM). Three milliliters of mobile phase (acetonitrile:water = 60:40) were added to the extracted PTX after the DCM had evaporated.[14] The resulting solution was filtered into a vial for HPLC (Aglient 1100, USA) detection of the PTX concentration. To detect PTX, a HPLC system was employed utilizing the C18 column (150 $\times$ 46 mm^2, 5 μm). The mobile phase comprised of acetonitrile and water (60/40, v/v)[23]; the injection volume was set at 20 μL; and the flow rate was fixed at 1.0 ml/min. The column effluent around at 4.6 min was detected at 227 nm. The calibration curve for the PTX was linear over the concentration range of 2.7225–76.23 ppm with a correlation coefficient of R^2 = 0.9999. The solvent for calibration was the mobile phase.

The encapsulation efficiency of PTX was determined by dissolving approximately 10 mg of fibers in 1 mL DCM in a screw cap tube. Three milliliters of mobile phase were added to the extracted PTX after the DCM had completely evaporated. The resulting solution was analyzed by HPLC method to determine the amount of PTX encapsulated in the microfiber samples.

Cell culture and cytotoxicity studies

The HeLa cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 units/mL streptomycin in humidified incubator with 5% CO_2 content at 37°C, and the culture medium was replaced by fresh RPMI-1640.

Previous work[24] demonstrated that PTX can effectively kill HeLa cells *in vitro*. The cytotoxicity of PTX was determined by an 3-[4,5-dimehyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. MFs were deposited on the coverslips (14 mm in diameter) and dried in a *vacuum* oven for a week to remove the residual solvents. Then the MFs were fixed in 24-well plate with stainless ring and decontaminated under UV light for 6 h before cytotoxicity test. The plates were incubated for 24 h, 48 h, 72 h, and 96 h. One row of the 24-well plate was used as control without adding fibers. One row was administered by blank P(LLA-CL) fibers. Other rows were administered by MFs prepared with different inner flow rates. About 200 μL of HeLa cells were transferred to 24-well plate first to ensure 2 $\times$ 10^4 cells per well. Medium was changed every other day. The well plates were incubated in a humid atmosphere at 37°C and 5% CO_2. The percentage of viable cells for each test was determined by measurement of MTT absorbance, relative to the final cell population in nontreated cultures. After incubation at given time, all medium were removed from the well. Then, 400 μL of the fresh medium without fetal bovine serum and 40 μL of MTT assay were added to each well. Following incubation for around 4 h, the solution was removed, then leaving the precipitate. Four-hundred microliters of DMSO were then added to the wells before the plate was observed using microplate reader. Finally, the optical density (OD) of the solution was measured by Enzyme-labeled instrument (MK3, Thermo, USA) at 492 nm. The OD values in one group were averaged. The relative cell viability rate was calculated by dividing the OD value of the test group by that of the control group.

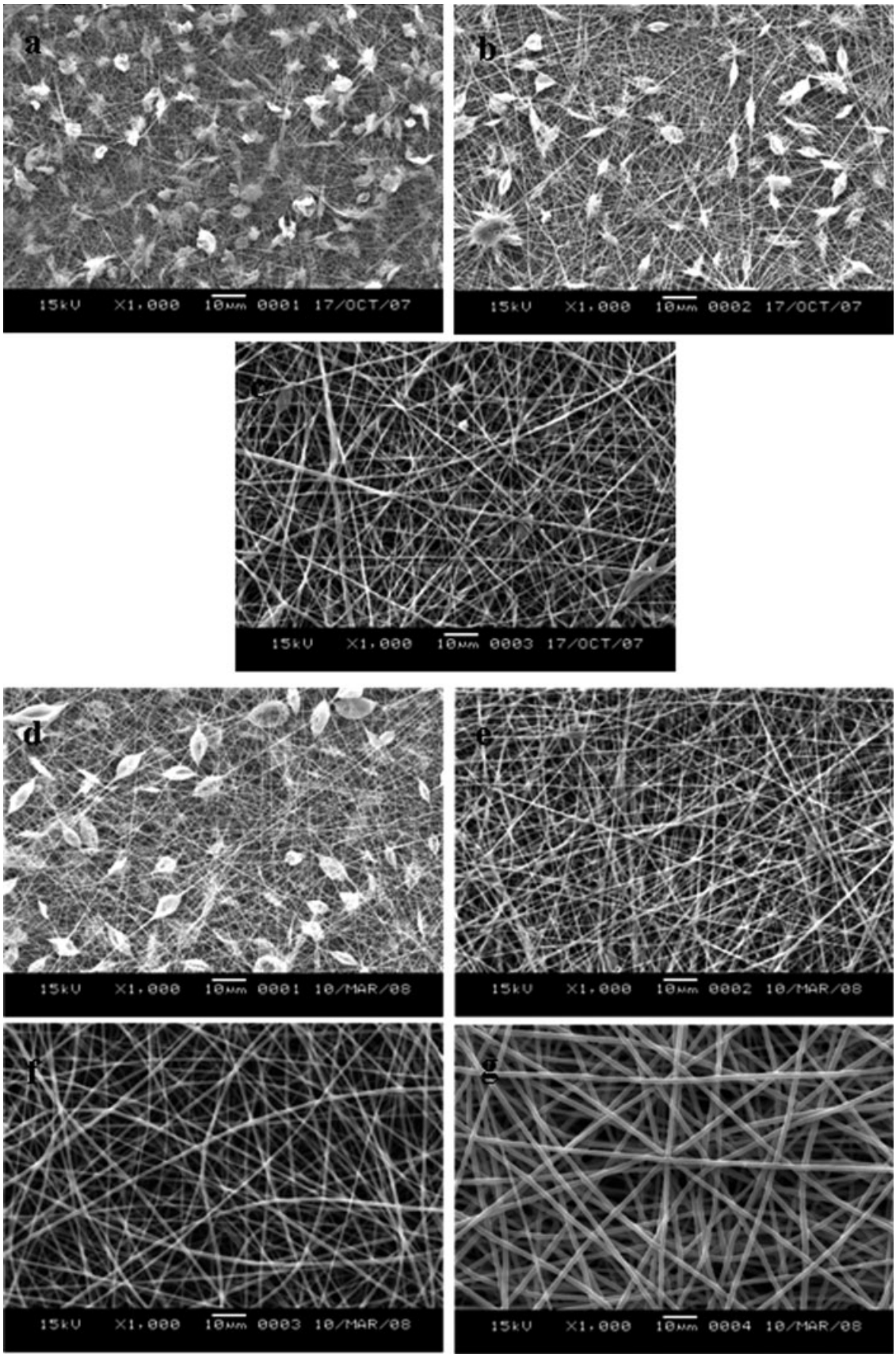

Figure 2. SEM images of coaxial electrospinning fibers of P(LLA-CL)(shell)/PTX(core) under different P(LLA-CL) concentrations and electrospinning conditions. (a–c) Electrospun shell/core ratio 3:1: (a) 2 wt %, (b) 4 wt %, (c) 6 wt %; (d–g) electrospun shell/core ratio 2:1: (d) 4 wt %, (e) 6 wt %, (f) 8 wt %, (g) 10 wt %.

Statistical analysis

All the data were obtained at least in triplicate and expressed as means ± standard deviation (SD). One-way ANOVA at a significance level of $p < 0.05$ was performed using Origin 7.5 (OriginLab Inc., USA).

RESULTS AND DISCUSSION

Morphology

The polymer concentration and the feed rate of the inner PTX solution have a crucial effect on membrane morphology, which is shown in Figure 2. The SEM and TEM photographs of MFs shown in Figures 2–4, were all electrospun with the same electrical voltage 12.5 kV and collecting distance 19 cm between the tip and the collector. The average diameters of the fibers with different concentrations were listed in Table I.

TABLE I
Diameters of Electrospinning PTX-Loaded P(LLA-CL) Microfibers

P(LLA-CL) concentration	4 wt %	6 wt %	8 wt %	10 wt %
Diameter mean (nm) ± SD	233 ± 68	503 ± 144	907 ± 172	1459 ± 150

When P(LLA-CL) concentration was 2 wt %, the membrane displayed bead-on-siring morphology [Fig. 2(a)]. With an increase in P(LLA-CL) concentrations, the defect density gradually diminished. When P(LLA-CL) concentration was above 8 wt %, MFs with no bead defects were obtained [Fig. 2(f,g)]. Figure 2(a–c) and (d–g) shows fibers from different inner feed rates. With increased inner feed rate, there was an associated increase in the bead defect density.

Figure 3 shows that the diameters of electrospinning MFs could be adjusted by change in the feed

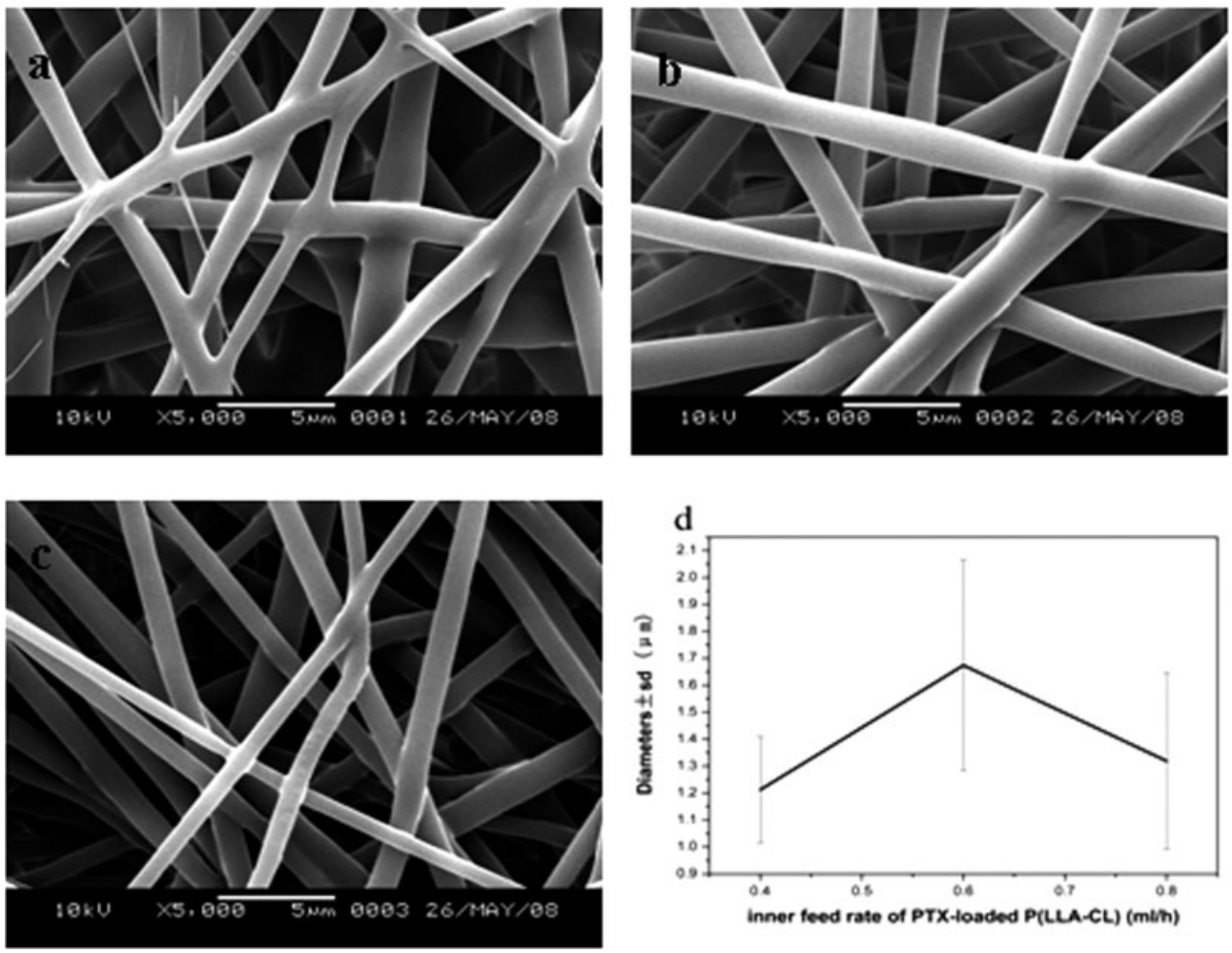

Figure 3. SEM photographs of MF of Paclitaxel inner feed rate: (a) 0.4 mL/h (MF1), (b) 0.6 mL/h (MF2), (c) 0.8 mL/h (MF3), respectively. (d) The diameter for each sample was measured and averaged by Image J_1.34 ($n = 100$).

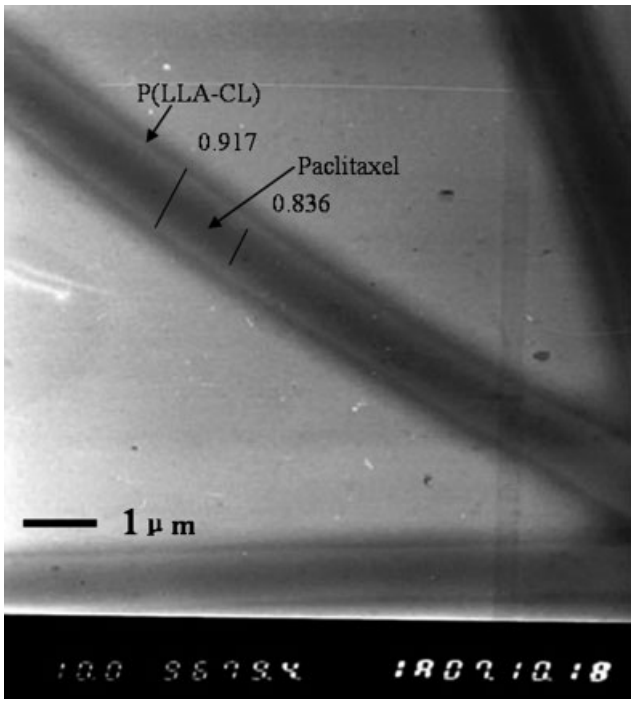

Figure 4. TEM photographs of microfibers.

rate of the inner dope. There was a corresponding increase in the diameter of electrospun fibers with an increase in the feed rate [Fig. 3(d)]. The diameters of MF with different inner feed rates were from 0.912 to 1.997 μm of 0.4 mL/h (MF1), from 0.656 to 2.226 μm of 0.6 mL/h (MF2), and from 0.610 to 2.353 μm of 0.8 mL/h (MF3). The results indicated that the diameter distribution of the fiber were influenced by the drug feed rate. No PTX crystals were detected by SEM, either on the surface of the fibers or outside the fibers, as seen in Figure 3(a–c). As shown in Figure 4, the core and the shell showed a sharp interface, and a relatively smooth core-shell interface was demonstrated. In other words, the encapsulation efficiency of MF was very well. Thus, both the shell solutions and the core solutions can influence the surface quality and morphology of the resulting MFs.[25]

XRD

The XRD spectra of pure PTX, P(LLA-CL) as-received, and MF for different inner feed rate from 0.4 to 0.8 mL/h were shown in Figure 5. PTX exhibited several intense peaks at $2\theta = 5.539^\circ$, 8.880°, and 12.260° in Figure 5(f). However, these peaks were not observed in the XRD patterns from samples of resulting fibers. The intensity of the XRD peak depends on the crystal size. Thus, the diffractograms of MFs indicated that, the drug would be either molecularly dispersed within the polymer or distributed in an amorphous state or crystalline with very small size.[26]

FTIR

Transmission infrared spectra of PTX powder, P(LLA-CL) as received, and MF were acquired to gain information on the molecular state of P(LLA-CL) and PTX. In the infrared spectrum, powder PTX exhibited a broad peak at 3510 cm^{-1}, which is assigned to the N—H and hydrogen bonded O—H stretch vibrational frequencies. The C=O ester and ketone stretch peak was present in the spectra at 1735 and 1712 cm^{-1}, and the C=O stretch of amide bond was observed at 1646 cm^{-1}. In Figure 6(b), spectrum of the P(LLA-CL) as received did not show significant differences at these peak. Moreover, it was shown that two peaks in Figure 6(a) fingerprint region could not be found in Figure 6(b). However, only one characteristic peak of PTX, at 710 and 765 cm^{-1}, which is assigned to the phenyl of C—H out-of-plane bending vibration, were observed in MFs, whereas other peaks were not discernible due to interference caused by polymers.

In vitro drug release

PTX is an important chemotherapeutic drug in treating tumors and other forms of cancer. The drug loadings for MFs were 7%, 10.9%, and 11.8% for theoretical drug loadings of 7.3%, 11%, and 13.7%, respectively. The encapsulation efficiency was more than 80%.

PTX was released from P(LLA-CL) MFs followed by a very slow release over the following 60 days. Figure 7 showed the release profiles of PTX from different inner feed rate 0.4 mL/h, 0.6 mL/h, and 0.8 mL/h of MFs, respectively. Obviously, in the whole drug release period, the release rate of PTX

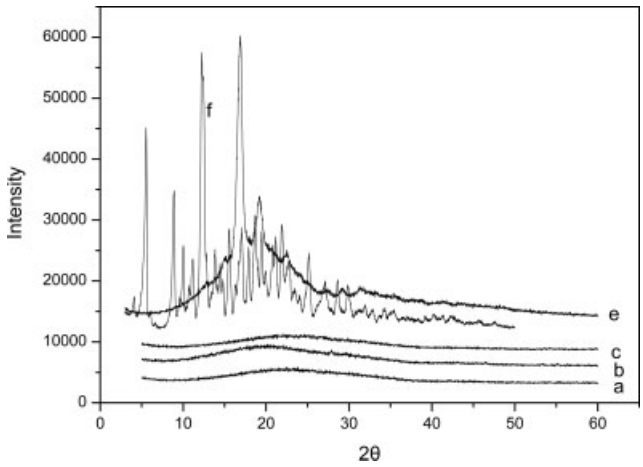

Figure 5. XRD of (a) MF1, (b) MF2, (c) MF3, (d) P(LLA-CL) as received, (e) pure Paclitaxel.

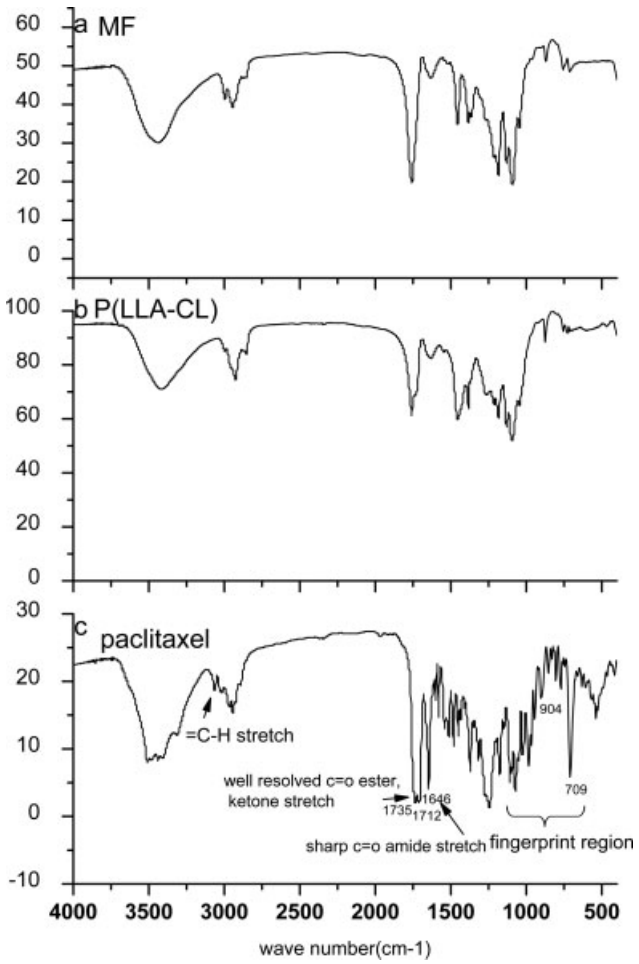

Figure 6. FTIR spectra of (a) MF, (b) P(LLA-CL) as received, (c) paclitaxel. All spectra are plotted in transmittance mode.

increased with increasing PTX feed rate. For example, their release percentages were about 48%, 27%, and 24% at 24 h, respectively, for the three samples examined. Initial burst release (with 1 h) mainly due to the diffusion of PTX near the fiber surfaces decreased with increasing PTX feed rate (30% of MF1, 16% of MF2, and 14% of MF3 at 1 h). The accumulative amount of PTX released from microfiber1 after 5 days was about 72%. It was about 93% for MF1 after 23 days. The release profiles of PTX from MF1 were quite different and characterized by the study with very slow or negligible release after 23 days. The accumulative amount of PTX released from MF2 after 5 days was about 38%. It was about 39% for MF2 after 60 days. The accumulative amount of PTX released from MF3 after 5 days was about 31%. It was about 33% for MF3 after 60 days. It is observed that the total amount of PTX release seemed to be less than 40% of the total amount of drug in the MF2 and the MF3 in 60 days.

However, the mechanism of PTX release from the copolymer matrices would involves[27,28]: (1) PTX diffusion through the polymer matrix and release into the media; (2) the erosion and swelling of the polymer matrix; (3) the degradation of polymer; (4) PTX dissolution/partitioning. In this study, the mechanism of PTX release mainly via a PTX diffusion through the pores of the polymer because P(LLA-CL) degrades quite slowly and more slowly within an *in vitro* medium without any enzymes. In this study, the relative rate of drug release is fastest with the lowest drug-loaded contents and it is slowest with the highest drug-loaded contents. A similar observation was reported by Deng et al.,[29] when they studied PTX-loaded methoxy poly(ethylene glycol)-*b*-poly(L-lactic acid) copolymer nanoparticles. In this study, PTX is highly hydrophobic (water insoluble, with water solubility less than 0.5 mg/L). When the inner feed rate increased, the affinity of P(LLA-CL) to hydrophobic PTX was greatly improved. Moreover, along with the drug-loaded increased, the inner core of drug disperse phase size and the drug crystal were also increased. In addition, the diameter of single fiber was also an important factor for the release rate of PTX. MFs electrospun from the inner feed rate of 0.4 mL/h were the smallest in size (see Fig. 3), the release of PTX from this sample was fastest. Therefore, the release rate of PTX from P(LLA-CL) MFs decreases with increasing drug-loaded content.

Cytotoxicity tests

The *in vitro* antitumor activity of the polymer/PTX nanoparticles or micelles against the Hela cells was

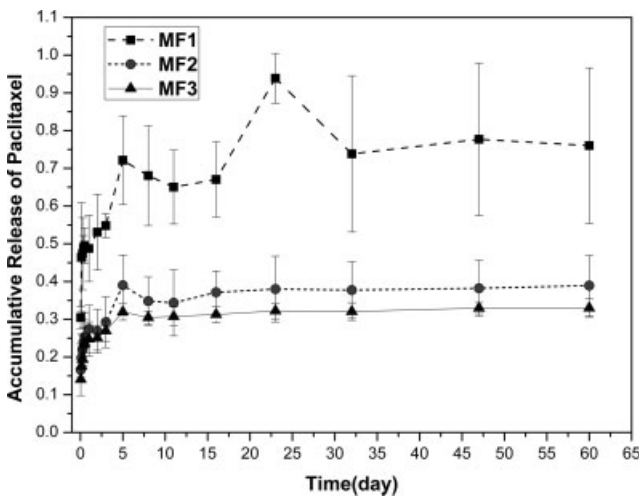

Figure 7. Time course of paclitaxel release from 75:25 P(LLA-CL) microfibers in PBS, pH 7.2 at 37°C (data are mean ± SD, $n = 3$). Films were fabricated at different inner feed rates. All date are representatives of three independent experiments.

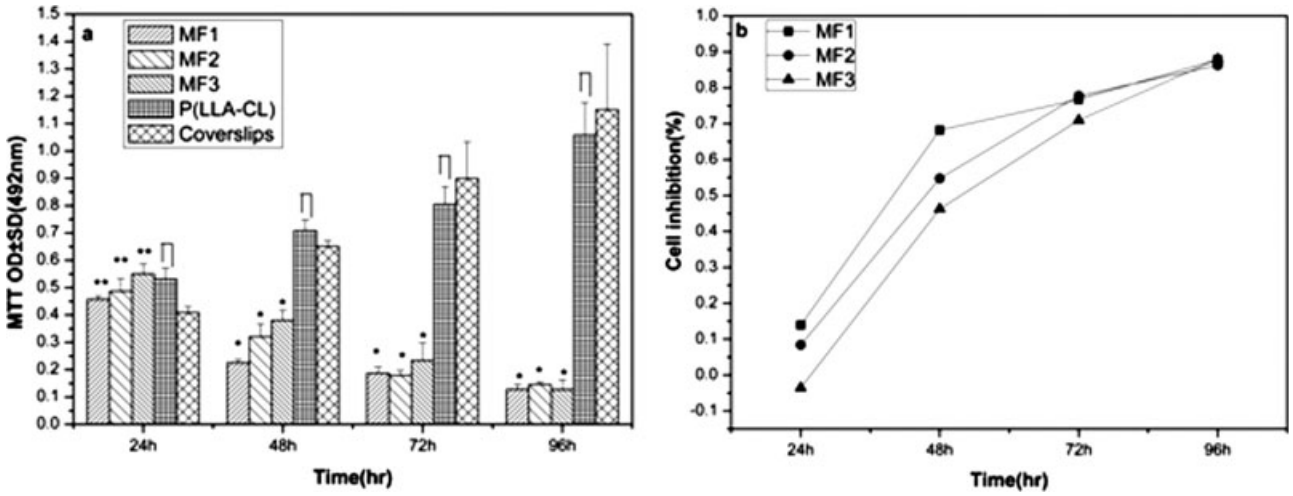

Figure 8. Cytotoxicity of the HeLa cells cultured on P(LLA-CL), MF, and coverslips. $^{**}p < 0.05$ and $^{*}p < 0.01$ relative to P(LLA-CL) fibers (mean ± SD, $n = 3$ in each group). Data are representatives of three independent experiments.

evaluated using MTT method. The cytotoxicity of the MF were shown in Figure 8. As shown in Figure 8(a), blank P(LLA-CL) fibers without PTX, the sample did not display any cytotoxicity to the HeLa cells up to 96 h. At 24, 48, 72, and 96 h, cell proliferation on all PTX-loaded P(LLA-CL) MFs were in lower speed compared with that on the coverslip and blank P(LLA-CL) MFs. In the case of MF1, MF2, and MF3, cell growth inhibition rates of 14%, 8%, and −3% were achieved, respectively, at 24 h [Fig. 8(b)]. In the case of MF1, MF2, and MF3 ($p < 0.01$), cell growth inhibition rates of 68%, 55%, and 46% were achieved, respectively, at 48 h [Fig. 8(b)]. And these values become 88%, 86%, and 88%, respectively, at 96 h [Fig. 8(b)]. In the case of MF1, MF2, and MF3 ($p < 0.01$), the release amount of PTX was enough to inhibit the cells, so the cell numbers decreased rapidly during the test. It implied that the drug lost its anticancer activity at 24 h, because of its own instability under the conditions or lower PTX release accumulation than other treatments. Fibers loaded with more PTX showed weaker cell growth inhibition before 96 h.

From these results, we can conclude that the PTX is continuously released with keeping their activity intact from the P(LLA-CL) MFs and the shortcomings of PTX such as instability in ambient atmosphere.

CONCLUSION

Coaxial electrospinning was successfully used to fabricate MFs with higher drug encapsulation efficiency. The sustained release of MF2 and MF3 could last for more than 60 days. The release rate of PTX from the fibers was dependent on the initial PTX loading. The release of PTX from the fibers followed diffusion and polymer erosion mechanism. The PTX release rate and the accumulation release decreased along with increase of the drug-loaded concentration and the hydrophobic chain in the copolymer. The cytotoxicity of PTX-loaded biodegradable MFs was found to be effective to inhibit HeLa cells growth, which evaluated MTT method test. These findings indicate that P(LLA-CL) fibers may be promising vehicles for hydrophobic drugs in delivery systems.

References

1. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly in vitro by Taxol. Nature 1979;277:665–667.
2. Feng S, Huang G. Effects of emulsifiers on the controlled release of paclitaxel (Taxol) from nanospheres of biodegradable polymers. J Control Release 2001;71:53–69.
3. Singla AK, Garg A, Aggarwal D. Paclitaxel and its formulations. Int J Pharm 2002;235:179–192.
4. Ceruti M, Crosasso P, Brusa P, Arpicco S, Dosio F, Cattel L. Preparation, characterization cytotoxicity and pharmacokinetics of liposomes containing water-soluble prodrugs of Paclitaxel. J Control Release 2000;63:141–153.
5. Perez-Stable C. 2-Methyoxyestradiol and paclitaxel have similar effects on the cell cycle and induction of apoptosis in prostate cancer cells. Cancer Lett 2006;231:29–64.
6. Kim SC, Kim DW, Shim YH, Bang JS, Oh HS, Wan Kim S, Seo MH. In vivo evaluation of polymeric micellar paclitaxel formulation: Toxicity and efficacy. J Control Release 2001;72: 191–202.
7. Zeng J, Xu X, Chen X, Liang Q, Bian X, Yang L, Jing X. Biodegradable electrospun fibers for drug delivery. J Control Release 2003;92:227–231.
8. Pan J, Feng SS. Targeted delivery of paclitaxel using folate-decorated poly(lactide)-vitamine E TPGS nanoparticles. Biomaterials 2008;29:2663–2672.
9. Park JS, Han TH, Lee KY, Han SS, Hwang JJ, Moon DH, Kim SY, Cho YW. *N*-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles for intracytoplasmic delivery of drugs: Endocytosis, exocytosis and drug release. J Control Release 2006;115:37–45.

10. Wang J, Ng CW, Win KY, Shoemakers P, Lee TK, Feng SS, Wang CH. Release of paclitaxel from polylactide-*co*-glycolide microparticles and discs under irradiation. J Microencapsul 2003;20:317–327.
11. Ciach T. Microencapsulation of drugs by electro-hydrodynamic atomization. Int J Pharm 2006;324:51–55.
12. Jiang H, Hu Y, Li Y, Zhao P, Zhu K, Chen W. A facile technique to prepare biodegradable coaxial electrospun nanofibers for controlled release of bioactive agents. J Control Release 2005;108:237–243.
13. Chew SY, Wen J, Yim EK, Leong KW. Sustained release of proteins from electrospun biodegradable fibers. Biomacromolecules 2005;6:2017–2024.
14. Xie J, Wang CH. Electrospun micro- and nanofibers for sustained delivery of paclitaxel to treat C6 giloma in vitro. Pharm Res 2006;23:1817–1828.
15. Li Y, Jiang H, Zhu K. Encapsulation and controlled release of lysozyme from electrospun poly(ε-caprolactone)/poly (ethylene glycol) non-woven membranes by formation of lysozyme-oleate complexes. J Mater Sci Mater Med 2008;19: 827–832.
16. Xu X, Chen X, Xu X, Lu T, Wang X, Yang L, Jing X. BCNU-loaded PEG-PLLA fibers and their in vitro antitumor activity against glioma C6 cells. J Control Release 2006;114:307–316.
17. Chen F, Li X, Mo XM, He CL, Wang HS, Ikada Y. Electrospun chitosan-(PLLA-CL) nanofibers for biomimetic extracellular marix. J Biomater Sci Polym Ed 2008;19:677–691.
18. Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: The road from laboratory to clinic, Part II (BMP delivery). J Tissue Eng Regen Med 2008;2:81–96.
19. Ayutsede J, Gandhi M, Sukigara S, Micklus M, Chen HE, Ko F. Regeneration of bombyx mori silk by electrospinning, Part 3: Characterization of electrospun nonwoven mat. Polymer 2005;46:1625–1634.
20. Bhattarai N, Edmondson D, Veiseh O, Matsen FA, Zhang M. Electrospun chitosan-based nanofibers and their cellular compatibility. Biomaterials 2005;26:6176–6184.
21. He CL, Huang ZM, Han XJ. Fabrication of drug loaded electrospun aligned fibrous threads for suture applications. J Biomed Mater Res A 2009;89:80–95.
22. Sanders EH, Kloefkorn R, Bowlin GL, Simpson DG, Wnek GE. Two-phase electrospinning from a single electrified jet: Microencapsulation of aqueous reservoirs in poly(ethylene-*co*-vinyl acetate) fibers. Macromolecules 2003;36:3803–3805.
23. Potineni A, Lynn DM, Langer R, Amiji MM. Poly(ethylene oxide)-modified poly(β-amino ester) nanoparticles as a pH-sensitive biodegradable system for paclitaxel delivery. J Control Release 2003;86:223–234.
24. Xie Z, Guan H, Chen X, Lu C, Chen L, Hu X, Shi Q, Jing X. A novel polymer-paclitaxel conjugate based on amphiphilic triblock copolymer. J Control Release 2007;117:210–216.
25. Han XJ, Huang ZM, He CL, Ling L, Wu QS. Coaxial electrospinning of PC(Shell)/PU(Core) composite nanofibers for texitile application. Polym Compos 2008;29:579–584.
26. Huang CY, Chen CM, Lee YD. Synthesis of high loading and encapsulation efficient paclitaxel-loaded poly(*n*-butyl cyanoacrylate) nanoparticles via miniemulsion. Int J Pharm 2007; 338:267–275.
27. Mu L, Feng SS. Vitamin E TPGS used as emulsifier in the solvent evaporation/extraction technique for fabrication of polymeric nanospheres for controlled release of paclitaxel (Taxol). J Control Release 2002;80:129–144.
28. Shi R, Burt HM. Amphiphilic dextran-graft-poly(ε-caprolactone) films for the controlled release of Paclitaxel. Int J Pharm 2004;271:167–179.
29. Deng LD, Li AG, Yao CM, Sun DX, Dong AJ. Methoxy poly-(ethylene glycol)-*b*-poly(L-lactic acid) copolymer nanoparticles as delivery vehicles for paclitaxel. J Appl Polymer Sci 2005; 98:2116–2122.