

RELEASE OF IBUPROFEN FROM PEG-PLLA ELECTROSPUN FIBERS CONTAINING POLY(ETHYLENE GLYCOL)-*b*-POLY(α -HYDROXY OCTANOIC ACID) AS AN ADDITIVE*

Hao Wang^{a,c}, Hong-rui Song^b, Xue-si Chen^{c**} and Ying-jie Deng^{a**}

^a School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

^b School of Pharmaceutical Engineering, Pharmaceutical University, Shenyang 110016, China

^c State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

Abstract Poly(α -hydroxy octanoic acid) was first used as an additive for the preparation of electrospun ultra-fine fibers of poly(ethylene glycol)-*b*-poly(L-lactide) (PEG-PLLA). Ibuprofen was loaded in the electrospun ultra-fine fibers. The results from environmental scanning electron microscopy (ESEM), wide angle X-ray diffraction (WAXD) and differential scanning calorimetry (DSC) demonstrated that ibuprofen could be perfectly entrapped in the fibers electrospun from PEG-PLLA using α -hydroxy octanoic acid or PEG-*b*-poly(α -hydroxy octanoic acid) (PEG-PHOA) as additives. Compared with electrospun PEG-PLLA fibers which entrapped 20 wt% ibuprofen, the PEG-PLLA electrospun fibers containing PEG-PHOA exhibited integral and robust after 1 week incubated in 37°C, pH 7.4 phosphate buffer solution with 10 μ g/mL proteinase K. Compared with electrospun fibers without PEG-PHOA, the concentration of proteinase K in release media had less effect on the release rate of ibuprofen. An unique release profile was found from PEG-PLLA fiber after the incorporation of PEG-PHOA. Enzyme degradation experiments demonstrated that PEG-PHOA but not α -hydroxy octanoic acid monomer was the crucial factor for integrity maintenance of the electrospun fibers, which may be due to the enzyme degradation tolerance property of the PEG-PHOA polymer additive.

Keywords: Poly(α -hydroxy octanoic acid); Poly(lactic acid); Electrospinning; Fiber; Ibuprofen; Drug release; Proteinase K.

INTRODUCTION

Biocompatible and biodegradable synthetic polymers such as poly(lactic acid) (PLA), poly(lactide-*co*-glycolide) (PLGA), poly(ϵ -caprolactone) (PCL)^[1–5] and their poly(ethylene glycol) (PEG) blocked copolymers^[6, 7] have been deeply studied and widely applied as carries of drugs. The delivery systems can exhibit controlled drug release behavior at definite time period or at targeted pathological site^[8, 9]. These polyester materials could degrade in physiology and pathology environment in a certain period of time^[10–13].

Due to the carbon-rich structure of the polymers, they are able to entrap water-insoluble compound at high efficiency thus satisfying the need of disease treatment by supplying sufficient therapeutic agents. However, due to their lack of chemical groups to be modified for adjusting release behavior of entrapped drugs, there have been already a lot of researches about PLA or PLGA physically commixed with other materials such as

* This work was financially supported by the National Natural Science Foundation of China (Nos. 20274048, 50373043), the Chinese Academy of Sciences (No. KJCX2-SW-H07) and the “863 project” (No. 2002AA326100).

** Corresponding author: Ying-jie Deng (邓英杰), E-mail: dengyingjie45@yahoo.com.cn

Xue-si Chen (陈学思), E-mail: xschen@ciac.jl.cn

Received April 3, 2009; Revised, August 10, 2009; Accepted September 3, 2009

doi: 10.1007/s10118-010-9041-x

polymers^[14–16], small molecule lipophilic compounds^[17] and even metals^[18] to improve the drug release system, and even chemically modified PLAs with functional groups were also used for drug delivery systems^[19–21]. Recently, Trimaille *et al.* reported a novel copolymer named PEG-*b*-poly(α -hydroxy octanoic acid) (short for PEG-PHOA) with the structure like *n*-hexyl attached to the main polyester chain^[22] of PLA. The structure of PEG-PHOA is shown in Fig. 1(A).

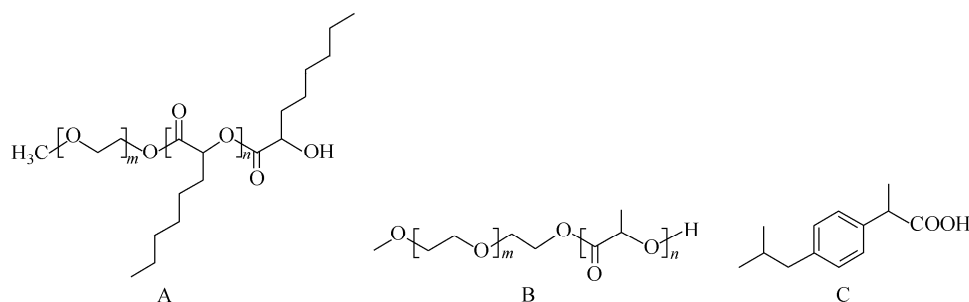


Fig. 1 Chemical structure of PEG-PHOA (A), PEG-PLLA (B) and ibuprofen (C)

Solution electrospinning is a kind of technique that can simply entrap many compounds into many kinds of polymer materials at high loading efficiency with fibers diameters at micro or even nanoscale^[23].

Due to the steric hindrance of hexyl side chain, it is difficult to obtain PHOA with high degree of polymerization^[24]. However PEG-PHOA with low degree of polymerization can not be used for electrospinning because organic solution of low molecular weight polymer has low viscosity not fitting for electrospinning. So low molecular PEG-PHOA was used as an additive for the electrospinning of high molecular weight PEG-PLLA. Medicated fiber mats constructed by this kind of polymeric materials presented gradually degradation property, which would satisfy the demand of prolonged therapy effect and fewer side effect, and further the polymer devices would disappear insensibly due to its biodegradability and thus increasing the compliance of patients^[25–27].

In our experiment, ibuprofen (IPF), the mostly used non-steroid anti-inflammatory drug was chosen as a model drug to be loaded into electrospun fibers. Lee *et al.* had studied the release profile of IPF entrapped in membranes composed of PEG modified PLLA^[28]. Polyethylene glycol (PEG) modification is a kind of method for modifying saturation and hydrophilicity of materials especially for polyesteroid which exhibits hydrophobic character resulting in its merely employment as medical devices^[29]. However, shortcoming also appears when PEG is copolymerized with polyester blocks like PLA and PLGA that PEG combines with H₂O easily leading to polyester degradation quickly in aqueous bio-environment. However, by our research, it was found that mixing PEG-PLLA fibers with PEG-PHOA could protect PEG-PLLA fibers from being degraded by proteinase K. Biodegradability of the fibers was investigated by incubating the fiber mats in PBS (phosphate buffer saline) added with 2.5, 5 and 10 μ g/mL proteinase K. ESEM results indicated that PEG-PHOA prohibited the fibers from being degraded in PBS solutions containing proteinase K. The release of ibuprofen from fibers was inhibited for the long biodegradation time of the hybrid fibers in PBS. This phenomenon was first discovered and reported in this work.

IPF entrapped fibers were successfully prepared by electrospinning. This novel medicated fiber mats might be useful in implantable local medical devices for treatment of post-operative dephlogisticate and cavity inflammation and so on.

MATERIALS AND METHODS

Materials

α -Hydroxy octanoic acid (α -HOA) was synthesized by the method reported in reference^[30]. Poly(ethylene glycol 2000 monomethyl ether)-*block*-poly(α -hydroxy octanoic acid) (PEG-PHOA) was also synthesized by the

method reported in reference^[24]. The weight-average molecular weight (M_w) of the obtained PEG-PHOA was 7470 with polydispersity index (PDI) 1.25. Ibuprofen was purchased from Juhua Group Pharmaceutical Factory, China (purity 98.7%). PEG-PLLA was synthesized by the ring-opening polymerization from L-lactide using poly(ethylene glycol) 2000 monomethoxyl ether purchased from Lancaster as initiator. The M_w of PEG-PLLA was 80400 with PDI 1.22. The M_w and PDI of the polymers were determined by gel permeation chromatography (GPC) on a USA Waters GPC combined with Wyatt DAWN EOS (MALLS) and DSP (RI) using chloroform as eluent. Proteinase K (biochemical degree) was purchased from Amresco and supplied as lyophilized powder. Triethyl benzyl ammonium chloride (TEBAC) was purchased from Sigma Aldrich.

Electrospinning

IPF, α -HOA, PEG-PHOA and PEG-PLLA were dissolved in chloroform forming homogeneous and transparent liquid solutions with appropriate viscosity which would facilitate electrospinning process and ensure the perfect incorporation of IPF in fibers.

PEG-PLLA was employed as main carrier materials. The hydrophilic block PEG has been widely employed in drug delivery system, and approved by the Food and Drug Administration (FDA) of USA for inner-body application^[31]. Low molecular weight PEG is readily excreted through the kidney. PEG block improved the hydrophilicity and degradation rate of PLLA copolymers, it also decreased the acidity of the degradation products, so that PEG-PLLA exhibits good potential for application in drug delivery systems^[32].

The mass ratios of spun materials were shown in Table 1. Generally, 20 wt% (respect to PEG-PLLA) IPF and 5 wt% (respect to materials) TEBAC were used in the experiments. TEBAC is electro-conducting substance necessary for electrospinning in the experiments. The whole solution was stirred for 24 h before electrospinning at room temperature to mix IPF and other materials thoroughly.

From Table 1, FM-1 (Fiber mat 1) is composed of PEG-PLLA, IPF and TEBAC. For FM-2 (Fiber mat 2), which was considered as objective sample for research, 5 wt% of PEG-PLLA was replaced by the same mass amount of PEG-PHOA. While 5 wt% of PEG-PLLA was replaced by the same mass amount of α -HOA for FM-3 (Fiber mat 3) as control FM sample.

Table 1. Mass amount of materials and drug used for electrospinning fibers in chloroform solution (mg)

Fiber mats	PEG2000-PLLA	PEG-PHOA	α -HOA	IPF	TEBAC
Fiber mat 1 (blank)	1500	0	0	300	75
Fiber mat 2 (objective)	1425	75	0	300	75
Fiber mat 3 (control)	1425	0	75	300	75

The electrospinning set-up was the same as reported by Xu *et al*^[33]. The copolymer and IPF blend was transferred to a 30 mL syringe attached with a right angle-shaped needle of 0.4 mm inner diameter. A pressure was applied to the solution in syringe to maintain a steady flow of the solution from the needle outlet in the range of 1.5–2.0 mL/h. The distance between the needle tip and the grounded target was 50 cm, and the electric field strength applied was 4500 V (90 V/cm). All electrospinning experiments were carried out at about 25°C in air. In order to remove the residual chloroform, the fiber mats collected were dried at 30°C under vacuum for 24 h.

Characterization of Fiber Mats

An environmental scanning electron microscope (ESEM, Model XL 30ESEM FEG from Micro FEI Philips) was used to observe the morphology of all the fibers. The electrospun fibers were mounted on glass slides by using a double-sided adhesive tape and vacuum-coated with an aurum layer prior to examination.

To investigate whether or not IPF was perfectly entrapped in the materials after electrospinning, the surface crystallization status of fiber mats were analyzed by wide angle X-ray diffraction (WAXD, Rigaku, D/max 2500V PC) at room temperature to detect the existing of crystalline substances. The samples were scanned from 5° to 60° at a scanning rate of 2 (°)/min.

Thermal parameters such as melting temperature (T_m) of IPF, TEBAC, α -HOA and glass transition

temperature (T_g) of PEG-PLLA powder and the fiber mats were determined by differential scanning calorimetry (DSC, Perkin Elmer) at a heating rate of 10 K/min from 0°C to 100°C under a N₂ atmosphere.

In Vitro Drug Release and Fiber Degradation

Drug release experiments were carried out by incubating pieces of drug loaded fiber mats of (50 ± 2) mg into 250 mL phosphate buffer solutions of pH 7.4 in a thermostated shaker of 37°C under oscillation at 100 r/min. For comparing the enzyme degradation effect on polymer fibers, IPF release behavior of the fiber mats was investigated in PBS with 2.5 µg/mL and 10 µg/mL proteinase K for polyester degradation. According to the Pharmacopeia of China, PBS was prepared by dissolving 6.8 g KH₂PO₄ and 1.58 g NaOH into deionized water and the solution was metered to 1000 mL. At predetermined time interval, 1 mL of release media of the fiber mat was withdrawn and analyzed by high performance liquid chromatography (HPLC). After this, 1mL release media was re-added into release media to maintain the same volume.

The HPLC system was conducted by using a Waters HPLC system equipped with a column (Dikma Diamond C18, ϕ 4.5 × 150 mm, Dikma), a UV-2487 UV-detector (Water) and a LC-10AT pump (Shimadzu). The eluent was composed by 80/20 mixture (V/V) of methanol and KH₂PO₄ deionized water solution (0.01 mol/L). The pH of the eluent was adjusted to 3.0 by phosphoric acid before use. The flow rate was 1.0 mL/min, and the detection wavelength was 225 nm. IPF is poorly water-soluble in pH 7 water but dissolved increasingly in aqueous solution with increasing pH. This facilitated the research process that the released drug could be determined by injecting release media into high pressure liquid system without organic solvent extraction procedure.

After 4 days drug release, residue in release media was taken out and washed by distilled water and freeze-dried for fibers degradation investigation.

RESULTS AND DISCUSSION

Electrospun Fibers Loaded with IPF

After eletrospinning, white, soft and continuous ibuprofen delivery devices of the ultra-fine fiber mats were obtained suitable for clinical application as implantable or stuffing devices. IPF was finely incorporated in the fibers and no IPF crystals were detected on the fiber surfaces. Under ESEM observation as shown in Fig. 2, the fiber surface of FM-2 exhibited slight crumples suggesting that monomer α -HOA had no effect while polymer PEG-PHOA had effect on the surface morphology. Diameter of medicated fibers with PEG-PHOA (FM-2) became bigger than those with α -HOA (FM-3) and without additives. Diameters of FM-2 were from 500 nm to 1 µm while FM-3 was from 300 nm to 500 nm. However, the fibers added with PEG-PHOA did not connect with each other at the crossing site compared with that of the fibers with α -HOA (FM-3).

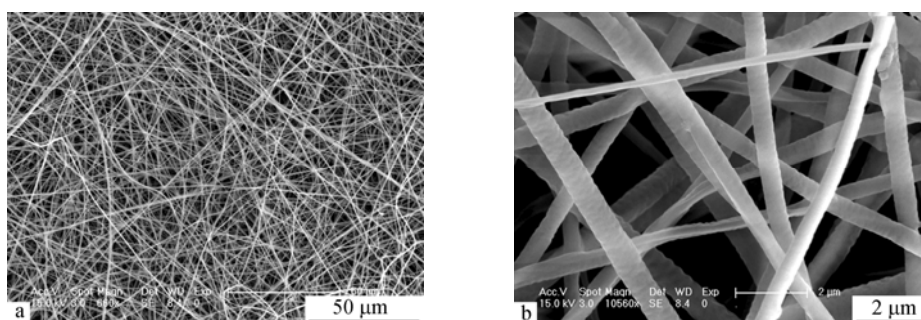


Fig. 2 ESEM of IPF loaded PEG-PLLA fibers with PEG-PHOA added (Fiber mat 2 in Table 1)

WAXD detection (Fig. 3) demonstrated that ibuprofen was perfectly entrapped in all of fiber mats for that IPF and PEG-PLLA peak disappeared. Addition of α -HOA and PEG-PHOA did not affect fibers from finely entrapping IPF. It was obviously that no phase separation took place during the rapid evaporation of the solvent

in the electrospinning process from the WAXD detection which was in accordance with the results from ESEM. Surfaces of all spun fibers were amorphous suggesting that IPF existed in amorphous form, probably as a solid dispersion or amorphous molecular aggregates on fiber surface. This would favor us analyzing the release pattern of IPF entrapped in the fibers.

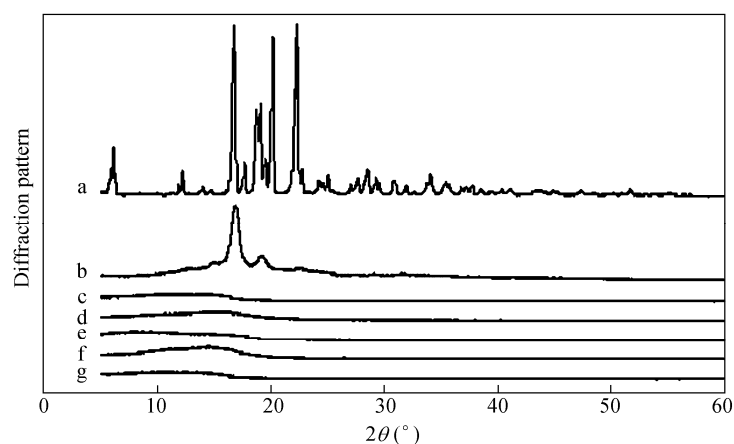


Fig. 3 WAXD patterns: IPF (a), PEG-PLLA (b), PEG-PLLA fibers without IPF and α -HOA (c), PEG-PLLA fibers with IPF and without α -HOA (d), PEG-PLLA fibers with PEG-PHOA (e), PEG-PLLA fibers with IPF and PEG-PHOA (f), PEG-PLLA fibers with IPF and α -HOA (g)

From DSC analysis of IPF, TEBAC, α -HOA, and fiber mats (Fig. 4), two pieces of important information could be seen that, first, PEG-PLLA fibers loaded with IPF with lower T_g demonstrated that better mobility of the molecular chains was obtained by incorporating IPF molecules; second, there was a tiny peak appeared at the position of melting point of IPF for FM-1 and FM-3 but not for FM-2, which revealed that 20 wt% IPF loading amount would lead to drug crystallizing in inner fiber, but PEG-PHOA could inhibit IPF crystallization in fibers.

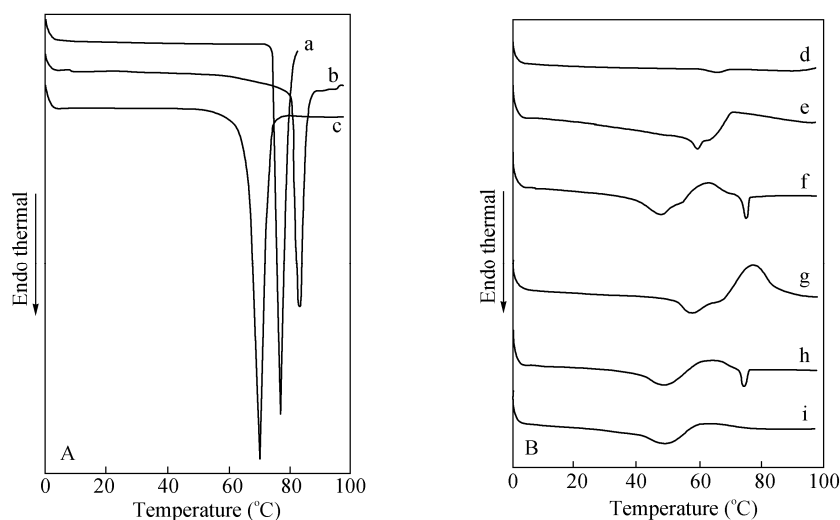


Fig. 4 DSC thermographs

A) IPF (a), TEBAC (b), α -HOA (c); B) PEG-PLLA (d), PEG-PLLA fibers without IPF and α -HOA (e), PEG-PLLA fibers with IPF and without α -HOA (f), PEG-PLLA fibers without IPF and with α -HOA (g), PEG-PLLA fibers with IPF and α -HOA (h), and PEG-PLLA fibers with IPF and PEG-PHOA (i)

IPF Release Profile from Fiber Mats

According to ESEM observation and WAXD detection, there was no crystalline IPF existing on the surface of any fibers investigated in our experiments, this demonstrated that IPF was perfectly entrapped in these materials or existing on fiber surface as amorphous status. Therefore, burst release was observed from release profile shown in Fig. 5. IPF was water-soluble to certain degree making it dissolved into release media rapidly.

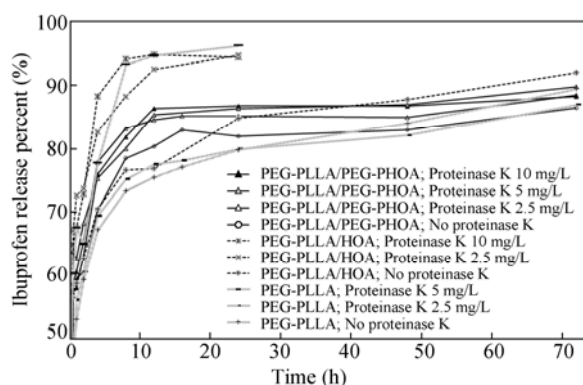


Fig. 5 Release profiles of IPF from FM1 (gray line), FM2 (black line), FM3 (dot line) in PBS with different concentrations of proteinase K ($n = 4$, RSD < 15%)

Fiber Degradation

There were 4 kinds of release media with different concentration of proteinase K (0, 2.5, 5 and 10 $\mu\text{g/mL}$) used for investigating the ability of fibers to the enzyme degradation.

Unique drug release pattern was found which could be clearly divided into two parts as burst release phase and sustained release phase. This provided a new approach for the drug stayed in the pathological site that the burst release satisfied the initial therapeutic agent supply and the sustained release subsequently afforded the release of rest drug. Generally, burst releases were observed in all fiber mats. 50% entrapped IPF was released in PBS from fibers without proteinase K, with 2.5, 5, or 10 $\mu\text{g/mL}$ proteinase K. IPF released more thoroughly from FM3 after 3-day degradation than from FM2. This demonstrated that α -HOA could not, but PEG-PHOA could prevent proteinase K from degrading fibers. This phenomenon revealed that α -HOA of polyester status was crucial for hindering proteinase K degradation effect.

Drug burst release was much more severe than that reported in other references which was mainly due to the PEG blocks facilitating IPF release from fibers. It was confirmed by ESEM and WAXD that there was no IPF crystals on fibers surface as shown in Fig. 2 and Fig. 3. The burst release was probably due to the existence of PEG-blocks in fibers which incorporated some IPF. When release media was added, IPF entrapped in PEG-blocks could be easily dissolved into release media. The other reason was that there was more IPF distributed near the fiber surfaces after electrospinning although IPF was uniformly distributed in electrospinning solution. The addition of α -HOA and PEG-PHOA did not solve the problem of IPF burst release.

Drug release behaviors were closely related to the distribution status of the drug. DSC detection shown in Fig. 4 demonstrated that some amount of crystal or semi-crystal IPF existed in fibers. Appropriate burst release may fit the delivery of anti-inflammatory drugs where a certain amount of initial burst is required to achieve enough exposure of drug to pathological site.

Although there was about 50 wt% initial burst release within 1 h in all the samples investigated regardless addition of α -HOA or PEG-PHOA or the concentration of proteinase K in release media. After 1 h, there were different IPF release behaviors for different fiber mats and in different release media with different concentrations of proteinase K. As shown in Fig. 5, IPF release rate from the fiber mats increased significantly as concentration of proteinase K increased from 0 $\mu\text{g/mL}$ to 10 $\mu\text{g/mL}$ for FM1 and FM3. For FM3, when concentration of proteinase K increased from 0 $\mu\text{g/mL}$ to 2.5 $\mu\text{g/mL}$, IPF release rate became significantly

faster, but for FM2, increase of IPF release rate did not change significantly. This phenomenon demonstrated that PEG-PHOA being entrapped in PEG-PLLA not only prohibited proteinase K from degradation of the fibers but also resulted in IPF being clutching tightly in fibers.

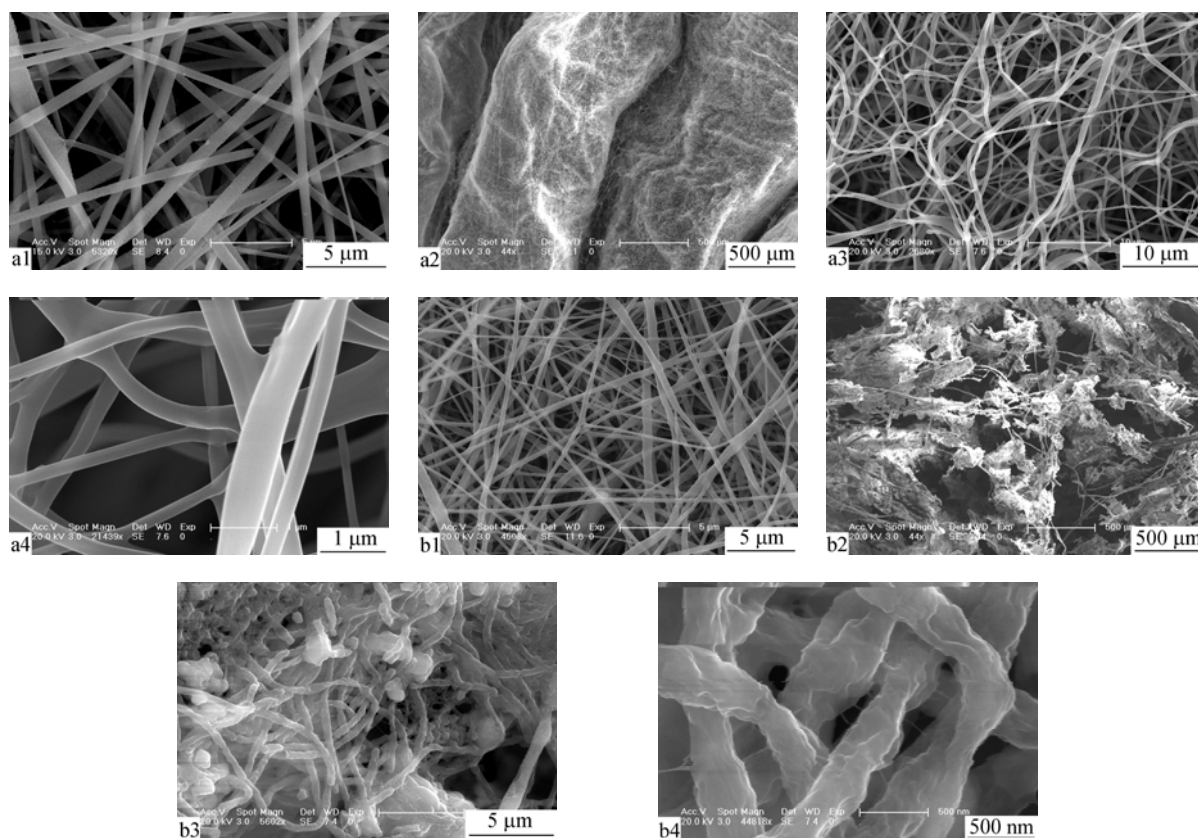
When the concentration of proteinase K in release media was 2.5 $\mu\text{g/mL}$, FM3 released IPF much faster than FM1, which demonstrated that the addition of monomer α -HOA in PEG-PLLA facilitated the release of IPF. It can be explained that α -HOA is water soluble so that pores is formed in fibers when the fiber is immersed in release media.

The drug release from the medicated fibers of degradable polyester followed a combined mechanism of diffusion and enzymatic degradation corrosion. At the early stage, the diffusion mechanism was predominant and a certain time later, the enzymatic degradation mechanism became predominant. So IPF release behavior from fibers could reflect the degradation status of fiber by enzyme.

As control experiment, the fibers commixed with poly(ϵ -caprolactone) (5 wt% respect to PEG-PLLA) of M_w 10000 were also investigated for fiber degradation. After being immersed in 10 $\mu\text{g/mL}$ PBS solution with proteinase K at 37°C for 72 h, fibers broke down into pieces and dissolved partly into release media. This confirmed the conclusion mentioned above.

Fibers of FM1 and FM3 presented faster release rate compared with FM2. Above 90% entrapped IPF was released from the fibers mats into release media when proteinase K in release media was 10 $\mu\text{g/mL}$ at 37°C after 24 h. However, for FM2 less than 90% entrapped IPF could be released at the same condition.

From Fig. 6, we could also see that Fiber mat 3 corroded severely into pieces and even dissolved in release media (b2, b3, b4 and c) while smooth surface was still retained in Fiber mats 2 after 1 week ibuprofen release in release media (a2, a3, a4 and c) containing 10 $\mu\text{g/mL}$ proteinase K. All of the apparent phenomena under ESEM were in accordance with the IPF release experiment.



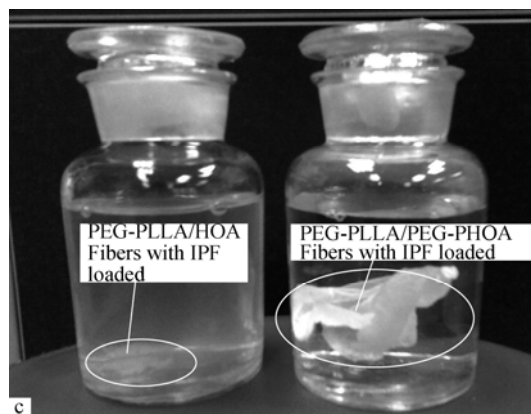


Fig. 6 Different magnification ESEM of fiber mats electrospun from IPF entrapped PEG-PLLA/PEG-PHOA fibers (a1) and PEG-PLLA/ α -HOA fibers (b1) and freeze-dried residue of a1 (a2, a3, a4) and b1 (b2, b3, b4) (bar represent 5 μ m in a1 and b1, 500 μ m in a2 and b2, 10 μ m in a3, 5 μ m in b3, 1 μ m in a4, 500 nm in b4) and appearance of two kind of fiber mats immersed in release media (c, in white circles)
Enzyme degradation condition: 1 week incubation in pH 7.4 PBS (added with 10 μ g/mL proteinase K) at oscillation of 100 r/min at 37°C

The reason for these phenomena is obviously due to the addition of PEG-PHOA additive to PEG-PLLA polymer. The cause of the unique release phenomenon of IPF from PEG-PLLA fibers may be attributed to the hexyl side chains in PHOA that hinder proteinase K from degrading ester bonds in PLLA.

CONCLUSIONS

In this research, PEG-PLLA fiber mats with finely entrapped ibuprofen using PEG-PHOA as additives were successfully prepared by electrospinning. It was confirmed that the crucial factor for integrity of fiber mats in PBS with high concentration of proteinase K was the polymer of α -hydroxy octanoic acid but not the monomer. From the results of *in vitro* fiber degradation and drug release experiment, we could clearly know that degradation of fiber and entrapped drug release profile in fiber could be tuned by adding a small amount of low molecular weight polymer to another high molecular weight polymer. Next research will be the modification of the degradation property of PEG-PLLA/PEG-PHOA hybrid fibers by decreasing the carbon number in PHOA to develop a more degradation-tolerant drug release systems.

At last, it should be also noticed that if other kinds of drug were chosen as the model compound, especially for those very water-soluble drugs and very water-insoluble drugs, the results might be probably different that the release behavior would be totally another one although the same polymer and additive were chosen. It is necessary to do much more work in the future to investigate the release character for other drugs.

REFERENCES

- 1 Biondi, M., Ungaro, F., Quaglia, F. and Netti, P.A., *Adv. Drug Deliv. Rev.*, 2008, 60: 229
- 2 Vasir, J.K. and Labhasetwar, V., *Adv. Drug Deliv. Rev.*, 2007, 59: 718
- 3 Kretlow, J.D., Klouda, L. and Mikos, A.G., *Adv. Drug Deliv. Rev.*, 2007, 59: 263
- 4 Saito, N., Murakami, N., Takahashi, J., Horiuchi, H., Ota, H., Kato, H., Okada, T., Nozaki, K. and Takaoka, K., *Adv. Drug Deliv. Rev.*, 2005, 57: 1037
- 5 Jilek, S., Merkle, H.P. and Walter, E., *Adv. Drug Deliv. Rev.*, 2005, 57: 377
- 6 Cerral, P., Tricoli, M., Lelli, L. and Guerra, G.D., *J. Mater. Sci. Mater. Med.*, 1994, 5: 308
- 7 Kissel, T., Li, Y. and Unger, F., *Adv. Drug Deliv. Rev.*, 2002, 54: 99

- 8 Anderson, J.M. and Shive, M.S., *Adv. Drug Deliv. Rev.*, 1997, 28: 5
- 9 Yasukawa, T., Kimura, H., Tabata, Y. and Ogura, Y., *Adv. Drug Deliv. Rev.*, 2001, 52: 25
- 10 Vila, A., Gill, H., McCallion, O. and Alonso, M.J., *J. Control. Release*, 2004, 98: 231
- 11 Hayashi, T., *Prog. Polym. Sci.*, 1994, 19(4): 663
- 12 Blanco, E., Bey, E.A., Dong, Y., Weinberg, B.D., Sutton, D.M., Boothman, D.A. and Gao, J.M., *J. Control. Release*, 2007, 122: 365
- 13 Choi, K.S. and Kim, D.J., *J. Appl. Polym. Sci.*, 2002, 83: 435
- 14 Xiong, X.Y., Li, Y.P., Li, Z.L., Zhou, C.L., Tam, K.C., Liu, Z.Y. and Xie, G.X., *J. Control. Release*, 2007, 120: 11
- 15 Xiong, X.Y., Tam, K.C. and Gan, L.H., *J. Control. Release*, 2005, 103: 73
- 16 Kasperczyk, J., Jelonek, K., Dobrzyński, P. and Jarz, B., *J. Control. Release*, 2006, 116: e5
- 17 Urata, T., Arimori, K. and Nakano, M., *J. Control. Release*, 1999, 58: 133
- 18 Ishihara, T., Izumo, N., Higaki, M., Shimada, E., Hagi, T., Mine, L., Ogawa, Y. and Mizushima, Y., *J. Control. Release*, 2005, 105: 68
- 19 Gao, H., Yang, Y.W., Fan, Y.G. and Ma, J.B., *J. Control. Release*, 2006, 112: 301
- 20 Lee, E.S., Oh, K.T., Kim, D., Youn, Y.S. and Bae, Y.H., *J. Control. Release*, 2007, 123: 19
- 21 Xie, Z.G., Guan, H.L., Chen, X.S., Lv, C.H., Chen, L., Xu, X.L., Shi, Q. and Jing, X.B., *J. Control. Release*, 2007, 117(2): 210
- 22 Trimaille, T., Möller, M. and Gurny, R., *J. Polym. Sci. Part A: Polym. Chem.*, 2004, 42: 4379
- 23 Kenawy, E.R., Bowlin, G.L., Mansfield, K., Layman, J., Simpson, D.G., Sanders, E.H. and Wnek, G.E., *J. Control. Release*, 2002, 81: 57
- 24 Trimaille, T., Mondon, K., Gurny, R. and Möller, M., *Int. J. Pharm.*, 2006, 319: 147
- 25 Zeng, J., Chen, X.S. and Jing, X.B., *J. Appl. Polym. Sci.*, 2003, 89: 1085
- 26 Zeng, J., Xu, X.Y., Chen, X.S., Liang, Q.Z., Bian, X.C., Yang, L.X. and Jing, X.B., *J. Control. Release*, 2003, 92: 227
- 27 Zeng, J., Yang, L.X., Liang, Q.Z., Zhang, X.F., Guan, H.L., Xu, X.L., Chen, X.S. and Jing, X.B., *J. Control. Release*, 2005, 105: 43
- 28 Lee, J.H., Go, A.K., Oh, S.H., Lee, K.E. and Yuk, S.H., *Biomaterials*, 2005, 26: 671
- 29 Zhu, K.J., Xiangzhou, L. and Shilin, Y., *J. Appl. Polym. Sci.*, 1990, 29: 1
- 30 Wang, H., Deng, Y.J., Wang Y.L. and Zheng, H., *Chinese Journal of Synthetic Chemistry*, 2008, 16(1): 53
- 31 Kim, K., Luu, Y.K., Chang, C., Fang, D.F., Hsiao, B.S., Chu, B. and Hadjiargyrou, M., *J. Control. Release*, 2004, 98: 47
- 32 Huang, Y.Y., Chung, T.W. and Tzeng, T.W., *Int. J. Pharm.*, 1999, 182: 93
- 33 Xu, X.L., Chen, X.S., Xu, X.Y., Lu, T.C., Wang, X., Yang, L.X. and Jing, X.B., *J. Control. Release*, 2006, 114: 307