Preparation and Characterization of the Electrospun Nanofibers Loaded with Clarithromycin

Anfang Wei,[1,2] Juan Wang,[3] Xueqian Wang,[2] Qufu Wei,[1,2] Mingqiao Ge,[1] Dayin Hou[2]

[1]*Key Laboratory of Eco-textiles, Ministry of Education, Jiangnan University, Wuxi 214122, People's Republic of China*
[2]*Textiles and Clothing Department, Anhui University of Technology and Science, Wuhu 241000, People's Republic of China*
[3]*Department of Pharmacology, Wannan Medical College, Wuhu 24100, People's Republic of China*

Received 4 October 2009; accepted 28 February 2010
DOI 10.1002/app.32363
Published online 19 May 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Electrospinning was applied to prepare the drug-loaded nanofibers for potential use in drug delivery and wound healing. Clarithromycin (CLM) was selected as the model drug, whereas poly(L-lactic acid) (PLLA) was used as the biodegradable and biocompatible polymer carrier. The low toxicity solvents were tested, and the morphology and structures of the nanofibers were investigated by scanning electron microscopy (SEM), Fourier transform infrared spectrometer (FTIR), and X-ray diffraction (XRD). PLLA and its composite of CLM were electrospun using the solvents of dichloromethane and acetone. SEM images showed that the diameters of the electrospun PLLA fibers were about 1000 nm, decreased to about 400 nm when 5 wt % CLM was loaded. With the increase of the amount the drug loaded, the diameters of the fibers gradually decreased and their distributions varied. The drug aggregates of any kind were not observed on the surfaces of the fibers. FTIR spectra revealed that CLM was incorporated into the macromolecular carrier of PLLA by formation of the hydrogen bonds but no new functional groups in the structure of the composite nanofibers were formed. XRD patterns indicated that the drug distributed in the composite nanofibers existed in the noncrystalline form. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 346–352, 2010

Key words: elctrospinning; drug delivery; X-ray diffraction; FTIR; morphology

INTRODUCTION

Clarithromycin (CLM) is a new kind of semisynthetic macrolide antibiotics, which has been widely used to treat the infection of clinical respiratory tract such as pneumonia, bronchitis, and tonsillitis.[1] It also has the advantage of treating gastric ulcer in combination with other drugs.[2] CLM has low bioavailability *in vivo* due to its poor water solubility and short half life. CLM can also lead to some severe side effects, such as nausea, diarrhea, and abdominal pain.[3,4] So, it is necessary to develop a new form of CLM to avoid these disadvantages. One promising approach is to incorporate CLM into the polymer carrier by electrospinning. The advantages of this method are not only improvement in the drug's bioavailability but also to decrease its side effects.[5,6]

Electrospinning is a process that uses a strong electrostatic field to draw a polymer solution into fine fibers.[7] A basic electrospinning apparatus usually consists of three major components: a high voltage power supply, a spinneret and a grounded collecting plate. When a viscous fluid is charged with a high voltage, the electrostatic force will draw the fluid into liquid jet. Because of the interaction between the jet and external electrostatic field and charge repulsion inside the jet, the charged jet undergoes a bending or whipping instability to stretch it thinner. Solvent evaporation from the filaments results in solid fibers with diameters in the range of nanometers to microns. In most cases, these fibers deposit randomly on the electrode collector forming the nonwoven nanofiber webs. Recently, this technique has attracted a great deal of attention due to its ability to produce continuous ultrafine fibers with high surface area and porous structure easily. Various polymers have been electrospun into nanofibers, which have great potential in many

Correspondence to: Q. Wei (qfwei@jiangnan.edu.cn).
Contract grant sponsor: Anhui Province funded research projects for Young Teachers in Universities; contract grant number: 2008jq1079.
Contract grant sponsor: Anhui Provincial Natural Science Research Project of Universities; contract grant number: KJ2010B025.
Contract grant sponsor: Excellent Scientific and Technological Innovation Team of Jiangsu Province; contract grant number: (2009–10).
Contract grant sponsor: Natural Science Foundation of Anhui Province; contract grant number: 070414192.
Contract grant sponsor: Key Natural Science Foundation for the Universities of Anhui Province; contract grant number: KJ2009A119.

areas, such as filtration,[8] fibrous webs for aerosol purification,[9] thin coatings for defense, and protection.[10] Because nanofibers can offer site-specific delivery of drugs to the body, they have the advantages in reducing the drug's side effects and improving the drug's bioavailability, and more than one drug can also be encapsulated directly into the fibers, they are particularly interesting for drug delivery systems. Kenawy et al.[11] studied drug delivery from PLA, poly(ethylene-co-vinyl-acetate) (PEVA), and a 1 : 1 blend of the two polymers electrospun from chloroform solution with tetracycline hydrochloride as the model drug. Kim et al.[12] incorporated hydrophilic antibiotic into poly(lactide-co-glycolide) to produce nanofiber nanofibers. However, as reported,[13–16] most studies have focused on the release regularity of loaded drugs, few have concerned the interactions of the drug and the macromolecule carriers. To dissolve the drug and the carrier well, chloroform is mostly used in electrospinning because of its good solubility and inertness, but this solvent is medium toxic, which can hurt the researchers in the process of electrospinning and its remains in the electrospun nanofibers can also lead to side effects. Therefore, it is important to use the lower toxicity or nontoxicity solvents in electrospinning, and to study the interactions between the drugs and the carriers are also important for the drug release at a controlled rate.

Biodegradable polymers are good candidates for applications in biomedical field because of their biocompatibility, their degradation, and mechanical properties.[17] In this study PLLA, was used to prepare the nanofibers loaded with CLM by electrospinning. Low toxic solvents of acetone and dichloromethane were used and their ratios and the effects on the morphology of electrospun fibers were investigated. The structures and morphology of the nanofibers were studied by scanning electron microscopy (SEM), Fourier transform infrared spectrometer (FTIR), and X-ray Diffraction (XRD).

EXPERIMENTAL

Materials

CLM (purity more than 99%) was provided by Xinyu Chemical Engineering (Zhenjiang, China) and stored in the refrigerator at −20°C. Its chemical structure is showed in Figure 1. Poly(L-lactide) (PLLA) (M_η= 83,000) was purchased from Bright China Industrial (Shenzhen, China). The PLLA with a higher molecular weight (M_η = 100,000) purchased from the same company was also used for comparing the formation of the electrospun fibers. P Acetone and Dichloromethane were obtained from Sinopharm Chemical Reagent (China) and were used without further purification.

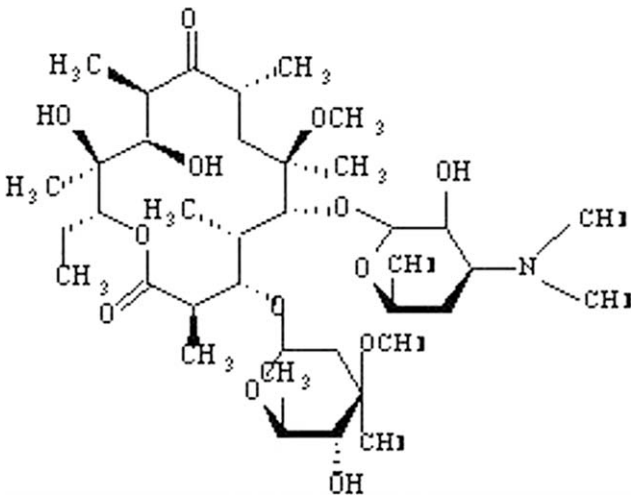

Figure 1 Chemical structure of clarithromycin.

Solvent selection

Acetone and dichloromethane, which are both low toxic solvents, were chosen in this work. The weigh ratios of these two solvents were varied from 1/1, 2/1, 1/2, 1/0, 0/1, and 1 to 0. Chips of PLLA (M_η = 83,000) were dissolved to prepare some certain concentration of the solutions. The ratio of acetone and dichloromethane was optimized by observing the morphology of electrospun nanofibers using SEM. To make further improvement in the elctrospinning quality, the larger molecular weight of PLLA (M_η=100,000) was selected to prepare the nanofibers. a weighed amounts of CLM power were then added into the above solvents system, which proportions (with respect to the polymer used) varied from 5, 10, 15, 30 wt %, were stirred for 3–4 h before electrospinning.

Electrospinning

The polymer solution was transferred to a 20 mL syringe with a flat-end metal needle, and then this syringe was set in a pump for controlled feeding rates. A high voltage direct-current (DC) power supply was used to initiate the jet and the electrospun nanofibers was collected onto a grounded cylindrical stainless steel mandrel. The electrostatic field was set at 13–15 kV and the distance between the needle tip and the collector was 18–22 cm. All electrospinning experiments were carried out at about 20°C in air. To remove the residual solvent, the fiber nanofibers collected were dried under vacuum at room temperature for 24 h. The control nanofibers were also fabricated by the same method without CLM incorporation.

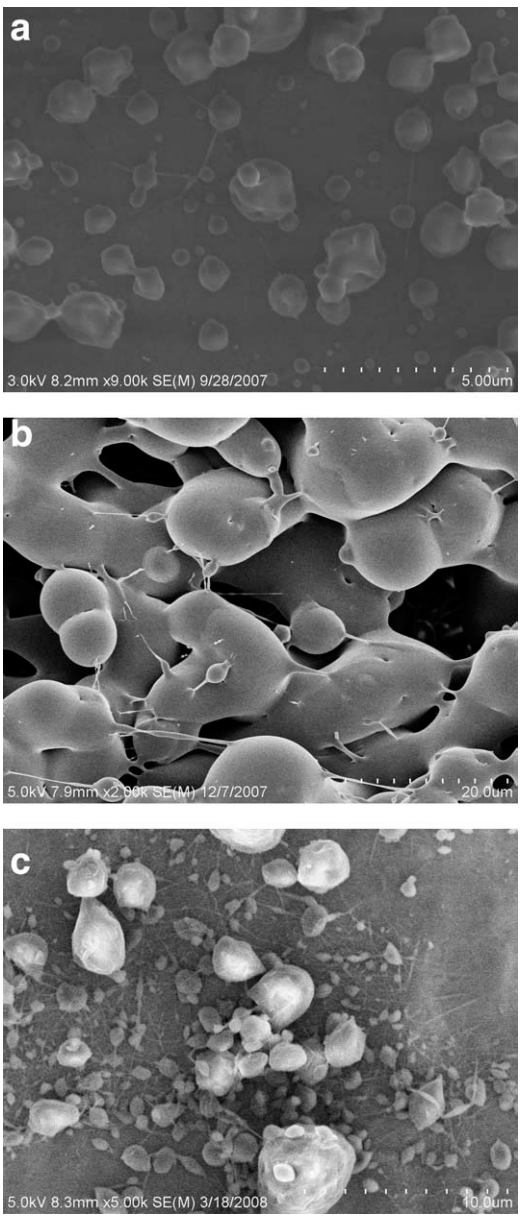

Figure 2 SEM photographs of electrospun PLLA (M_η = 83,000) in the acetone at the different concentrations: (a) 15, (b) 25, and (c) 30 wt %.

Characterization

The surface topography of the electrospun nanofibers was observed by a scanning electron microscope (Hitachi S-4800, Japan). The nanofibers were placed on a metal stud, fixed with double-sided conductive tape. A very thin layer of gold was applied to the fibers by a sputtering unit for 40 s. The gold-coated nanofibers were then placed in the microscope and scanned in 5 kV mode. The software of Image J was used to analyze the distributions of the electrospun fibers' diameters. CLM, the control and the composite nanofibers were prepared in KBr pellet and scanned by Fourier transform infrared spectrophotometer (FTIR, Nicolet NEXUS, and America). XRD patterns were obtained with a BRUKERS-AXS D 8 FOCUS Diffractometer. Scans were obtained in the 2θ range of 5–60° with a step size of 0.02° every 1 s.

RESULTS AND DISCUSSION

Morphology of the nanofibers

As is known, the solvents are very important factors in electrospinning. Based on the laboratory trials, the acetone and dichloromethanewe were chosen as the solvents and the different ratios of the two solvents were compared.

As illustrated in Figure 2(a), the collected material contained few nanofibers connected among the beads when only the solvent of acetone was used. The shape of beads changed from sphere to spindle and the numbers of fibers between the beads looked increased, but there were still a few when the PLLA concentration increased from 15 to 25%, as presented in Figure 2(b). The lower viscosity and high surface tension of the solution were easy to form beads in electrospinning.[18] PLLA and acetone were both polar and their interactions increased the surface tension of the solution. In addition, the viscosity-average molecular weight of PLLA (M_η=83,000) was lower, which also decreased the viscosity of the whole solution. Therefore, there were still less fibers formed even the PLLA concentration increased to 30%, as indicated in Figure 2(c).

As revealed in Table I, when some amount of dichloromethane was added into the solvent, the surface tension of the whole solution system decreased, which indicated that the polymer was easy to be stretched and form the nanofibers in

TABLE I
Solution Properties of 30 wt % PLLA (M_η = 83,000) in the Different Solvents

Different solvents (w w^{-1})	Surface tension (mN m^{-1})	Viscosity (mPa S)	Electrical conductivity ($\times 10^{-6}$ S m^{-1})
Acetone	30.60	11.0	13.6
Dichloromethane	26.61	45.0	0.8
Acetone/Dichloromethane (1/1)	27.48	20.0	8.2
Acetone/Dichloromethane (1/2)	26.35	22.5	5.9
Acetone/Dichloromethane (2/1)	27.51	16.5	9.4

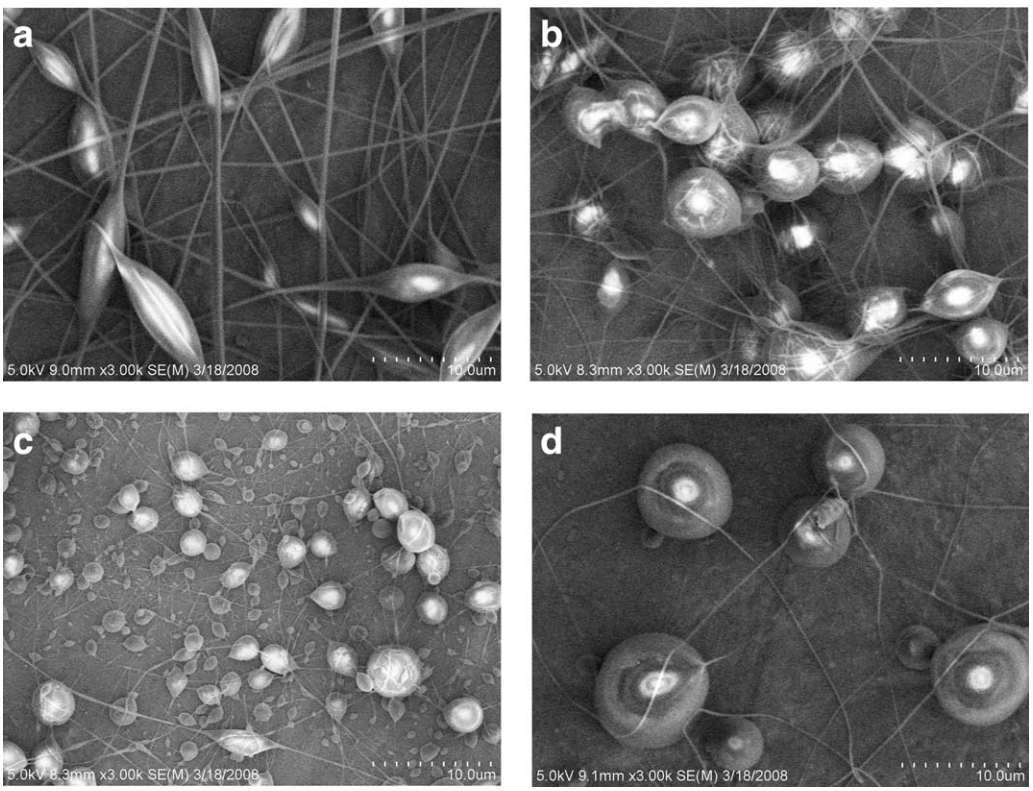

Figure 3 SEM photographs of electrospun PLLA (M_η= 83,000, 30 wt %) in the different solvents: (a) acetone/dichloromethane (1/2); (b)acetone/dichloromethane (1/1); (c) acetone/dichloromethane (2/1); and (d) dichloromethane.

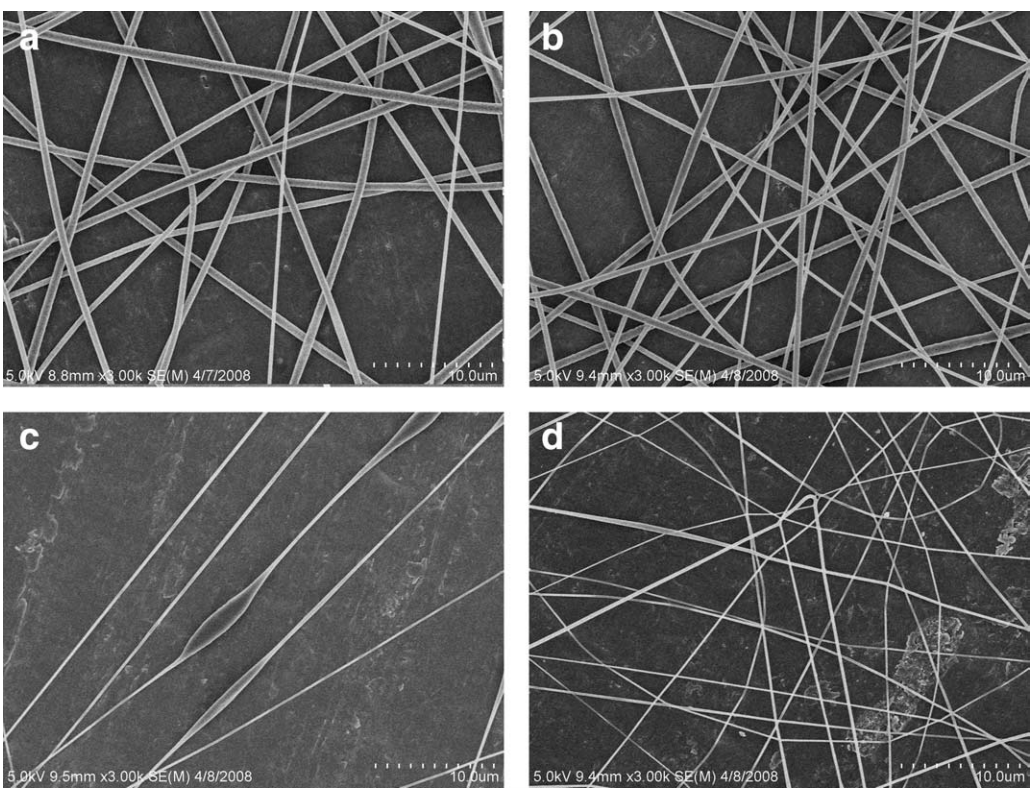

Figure 4 SEM photographs of PLLA (M_η= 100,000, 10 wt %) nanofibers containing (a) 0, (b) 5, (c) 10, and (d) 15 wt % of CLM.

TABLE II
Fiber Diameters With Different Amount of the Drug

Number	Drug loading (wt %)	Average fiber diameter (nm)	Standard deviation
1	0	1091.8	223.0
2	5	408.4	112.8
3	10	261.4	79.6
4	15	210.7	50.4

electrospinning. However, the electrical conductivity of dichloromethane was lower than that of acetone. The addition of dichloromethane also reduced the electrical conductivity of the whole solution, which would have some adverse effects on splitting of PLLA during electrospinning. The images in Figure 3 show the effect of solvent on the electrospinning. It was found that the nanofibers with a few beads were obtained when the ratio of acetone to dichloromethane was 1/2, as shown in Figure 3(a). It seemed that more beads were formed when the ratio of acetone to dichloromethane changed from 1/2 to 1/1 or 2/1, as revealed in Figure 3(b,c). The decrease in the electrical conductivity of the whole solution affected the splitting of the charged jet in the electrostatic field.

The nanofibers prepared by using PLLA (M_η= 100,000) and the ratio of acetone to dichloromethane at 1 : 2 are shown in Figure 4. It was observed that the PPLA with a higher molecular weight could form fibers without beads using the mixed solvents with a ratio of acetone to dichloromethane of 1 : 2. No presence of drug aggregates was observed on the surfaces of the electrospun fibers, as presented in Figure 4. It was also found that the fibers became finer when the amount of CLM was increased from 5 to 15%, as displayed in Figure 4(a–c). The diameters of the electrospun fibers analyzed by Image J are listed in Table II. The average diameter of PLLA control electrospun fibers was about 1091.8 nm, whereas the average diameter of the fibers loaded with 5 wt % CLM decreased to 408.4 nm. The average diameter of the electrospun fibers dropped further to about 261.4 and 210.7 nm as the drug loading amount was increased to 10 and 15%, respectively. It was also observed that the standard deviations of fiber diameter decreased, indicating evener distribution of the fiber diameters as the drug content was increased.

The concentration of PLLA in the solution was relatively reduced when PLLA was loaded with some amount of the drug, and its interaction with acetone was weakened so that the evaporation of acetone became easier. The addition of CLM not only decreased the concentration of PLLA in the solution, but also increased the interactions between the solvents and PLLA as CLM was also polar. The decrease in the viscosity of the solution and the increase in the surface tension led to the formation of thinner fibers and tangled fibers and more beads were easy to form as revealed in Figure 4, when the addition of CLM was increased.

FTIR spectra

As shown in Figure 5, both characteristic peaks of CLM and PLLA could be found in the FTIR spectra of the drug-loaded fibers. Some characteristic peaks migrated, but no other new peaks appeared in the FTIR spectra. The hydroxyl stretching peak of CLM was migrated from 3506.60 cm^{-1} to 3462.56 cm^{-1} and the carbonyl stretching peak of PLLA was also shifted from 1756.43 to 1752.39 cm^{-1} in the composite nanofiber nanofibers, which was caused by the formation of the hydrogen bond between the hydroxyl of CLM and the carbonyl of PLLA.[19] This strong action facilitated encapsulating the small molecules of CLM into the large molecules of PLLA. In fact, it was found the spectrum of the composite nanofibers was almost the same as that of PLLA when the FTIR-ATR (attenuated total reflection) was used to examine the chemical structure of the

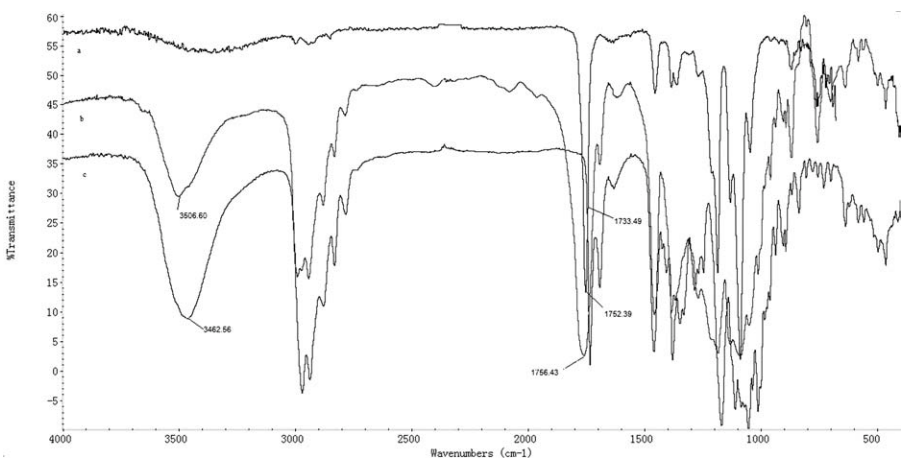

Figure 5 IR spectra of blank PLLA fiber (a), CLM (b), and PLLA nanofibers loaded with CLM (c).

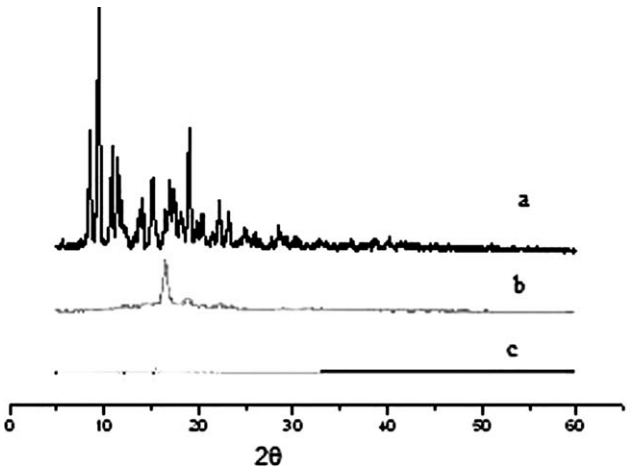

Figure 6 XRD patterns of (a) CLM, (b) cast films, and (c) PLLA nanofibers.

composite nanofibers. However, the spectrum revealed the characteristic peaks of the composite of PLLA and CLM when the KBr pellet method was adopted. This interesting phenomenon also indicated the small molecules of CLM maybe encapsulate into the large molecules of PLLA, which facilitated the controlled release of the drug in the drug delivery applications.

XRD Patterns

The XRD patterns of CLM, cast film of PLLA, electrospun PLLA nanofibers are presented in Figure 6. The characteristic peak of the cast film of PLLA was 16.6°, but the characteristic peak of PLLA electrospun nanofibers disappeared, which was consistent with the reports in some literatures.[20,21] There were many characteristic peaks on the XRD pattern of CLM, which indicated this drug was polycrystal.

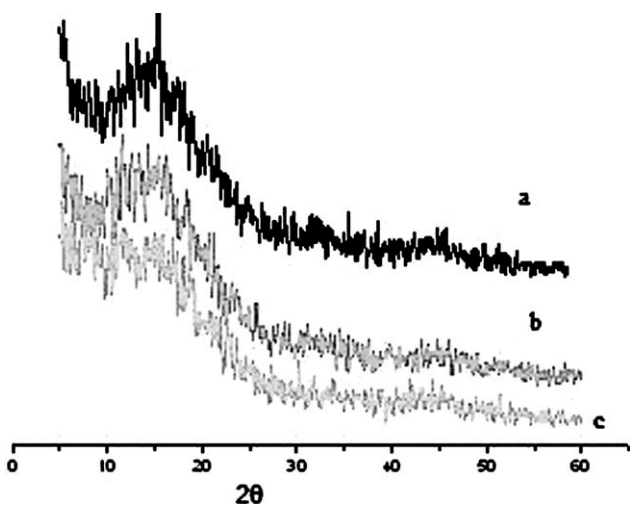

Figure 7 XRD patterns of PLLA nanofiber scaffolds containing (a) 0, (b) 5 wt %, and (c) 10 wt % CLM.

Electrospinning was such a fast process that molecular chains of PLLA could not crystallize in very short time. The XRD patterns of different content of CLM in PLLA nanofibers are shown in Figure 7. The variation of drug loading amount did not obviously change the pattern, which implied the dispersions of the drug were amorphous in PLLA, probably as a solid solution or amorphous molecular aggregates in the fibers. So, incorporation CLM into biodegradable PLLA nanofibers was feasible and the distribution state of the drug was amorphous, which developed the bioavailability of the drug and could have great potential for the drug release in the polymer carrier of PLLA nanofibers.

CONCLUSIONS

In eletrospinning, the viscosity, the surface tension, and the electrical conductivity of the spinning solution were the very important factors, which affected the formation of the nanofibers. Low toxic solvent system of acetone/dichloromethane (1/2) was selected in the preparation of the PLLA electrospun nanofibers loaded with CLM. When the loading amount was lower, such as 5 wt %, the diameters of electrospun fibers were smaller than the diameters of the control fibers. However, the distributions of the fibers tended to become evener with the increase of CLM loading amount. The FTIR spectra indicated the formation the hydrogen bond between the drug and PLLA in the composite nanofibers.

The XRD patterns of CLM/PLLA composite nanofibers revealed that CLM was incorporated in an amorphous state into the carrier of PLLA nanofibers. The study revealed that CLM/PLLA composite nanofibers could develop the bioavailability of CLM and control the release of CLM which was possibly encapsulated into the PLLA nanofibers for medical applications.

References

1. Chu, S.; Wilson, D. S.; Deaton, R. L.; Mackenthun, A. V.; Eason, C. N.; Cavanaugh, J. H. J Clin Pharmacol 1993, 33, 719.
2. Guay, D. R.; Gustavson, L. E.; Devcich, K. J.; Zhang, J.; Cao, G.; Olson, C. A. Clin Ther 2001, 4, 566.
3. Gaynor, M.; Mankin, A. S. Front Med Chem 2005, 2, 21.
4. Sibley, S. D.; Pedersen, J. A. Environ Sci Technol 2008, 42, 422.
5. Verreck, G.; Chun, I.; Peeters, J.; Rosenblatt, J.; Brewster, M. E. Pharm Res 2003, 20, 810.
6. Ngawhirunpat, T.; Opanasopit, P.; Rojanarata, T.; Akkaramongkolporn, P.; Ruktanonchai, U.; Supaphol, P. Pharm Dev Technol 2009, 14, 70.
7. Reneker, D. H.; Yarin, A. L. Polymer 2008, 49, 2387.
8. Ding, B.; Wang, M. R.; Yu, J. Y.; Sun, G. Sensors 2009, 9, 1609.
9. Kim, C.; Park, S. H.; Lee, W. J.; Yang, K. S. Electrochim Acta 2004, 50, 877.
10. Riboldi, S. A.; Sampaolesi, M.; Neuenschwander, P.; Cossu, G.; Mantero, S. Biomaterials 2005, 26, 4606.

11. Kenawy, E. R.; Bowlin, G. L.; Mansfield, K.; Layman, J.; Sanders, E.; Simpson, D. G.; Wnek, G. E. J Control Release 2002, 81, 57.
12. Kim, K.; Luu, Y. K.; Chang, C.; Fang, D.; Hsiao, B. S.; Chu, B.; Hadjiargyrou, M. J Control Release 2004, 98, 47.
13. Xu', X.; Chen, X.; Xu, X.; Lu, T.; Wang, X.; Yang, L.; Jing, X. J Control Release 2006, 114, 307.
14. Zeng, J.; Xu, X. Y.; Chen, X. S.; Liang, Q. Z.; Bian, X. C.; Yang, L. X. J Control Release 2003, 92, 227.
15. Zeng, J.; Yang, L.; Liang, Q.; Zhang, X.; Guan, H.; Xu, X.; Chen, X.; Jing, X. J Control Release 2005, 105, 43.
16. Kenawy, E. R.; Abdel-Haya, F. I.; El-Newehya, M. H.; Wnek, G. E. Mater Chem Phys 2009, 113, 296.
17. Gupta, B.; Revagade, N.; Hilborn, J. Prog Polym Sci 2007, 32, 455.
18. Fong, H.; Chun, I.; Reneker, D. H. Polymer 1999, 40, 4585.
19. Sheetal, S. J.; Tejraj, M. A. Polymer 2006, 47, 8061.
20. Deitzel, J. M.; Kleinmeyer, J.; Harris, D.; Beck Tan, N. C. Polymer 2001, 42, 261.
21. Lee, K. H.; Kim, H. Y.; Khil, M.; Ra, Y. M.; Lee, D. R. Polymer 2003, 44, 1287.