Effect of Protein-Loading on Properties of Wet-Spun Poly(L,D-lactide) Multifilament Fibers

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ABSTRACT: Protein-loaded multifilament fibers were fabricated by the wet-spinning method. The polymers which were tested included poly(L,D-lactide) [P(L,D)LA], L/D ratio 96/4 and poly(L,DL-lactide) [P(L,DL)LA], L/DL ratio 70/30. The polymers were dissolved in dichloromethane and bovine serum albumin (BSA) was dissolved in water, respectively. The solutions were mixed together using a probe sonicator to form a polymer-protein emulsion. This emulsion was extruded to an ethanol spin bath. The fibers possessed a distinct sheath-core structure, where the inner core was porous and the outer sheath was smooth. The diameters of the filaments were in the range of 46 and 70 μm. The tenacity values of the filaments were between 7 and 17 MPa. *In vitro* drug release rate of the P(L,DL)LA 70/30 filament was faster than that of the P(L,D)LA 96/4 filament. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2174–2180, 2010

Key words: biopolymers; degradation; fibers; proteins; mechanical properties

INTRODUCTION

Polymeric drug release devices enable the drug delivery over an extended period of time and to the local site of action. As a drug delivery device, fibers have a large surface area compared to volume, and thus a high capacity for drug release. Furthermore it is possible to fabricate different types of textile structures using the fibers, for example, knitted, woven, nonwoven, or braided structures having controlled porosity.

Textile structures made from fibers are suitable for scaffolds used in tissue engineering. The structures consist of an interconnected and permeable pore network which promotes nutrient and waste exchange of cells. A three-dimensional and highly porous structure also supports cell attachment, proliferation, and the formation of an extracellular matrix. Other advantages of textile structures are that they can be manufactured to have the appropriate mechanical strength needed by matrix for implantation, architecture which promotes formation of native anisotropic tissue, and reproducible architecture of clinically relevant size and shape.[1] Fibers are possible to fabricate for textile structures from polymers which are biocompatible and biodegradable with controllable degradation kinetics.

The release rate of the drug can be controlled by the choice of polymer and drug, and the design of device system. The factors influencing to the degradation of the polymer, and thus to the release of the drug, are polymer properties (molecular weight and its distribution, crystallinity, glass transition temperature, and melting temperature), the surface area of the sample, and the degradation conditions.

Polylactide is a biodegradable and biocompatible polymer, and it is widely used in medical applications. Enantiomerically pure polylactide, poly(L-lactide) [P(L)LA], is a semicrystalline polymer and its degradation time could be many years.[2] When a more rapid degradation rate is needed, it is possible to use polylactide stereo copolymers. The degradation time of polylactide can be controlled through the copolymerization of L-lactide with a different ratio to D-lactide or DL-lactide.[3] The increase in the D-lactide content in the copolymer increases the disorder in the polymer chains and reduces the crystallinity. During the hydrolytic degradation water diffuses into the amorphous regions of polymer and causes the breakage of the ester bonds which initiates a reduction in molecular weight and later on a reduction in

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mechanical strength. Thus the more amorphous polylactide stereo copolymers have a shorter degradation time. Veiranto et al.[4] have studied the release of ciprofloxacin (CF) from screws made from self-reinforced poly(L,DL-lactide) [P(L,DL)LA] 70/30, and they have observed the increasing concentrations of CF after 9 weeks *in vitro*; CF was totally released after 44 weeks *in vitro*.

The main production method of polylactide fibers is melt-spinning.[5] The polymer is heated until it melts, and the molten polymer is then forced through the spinneret holes. When the polymer jets emerge from the spinneret, they are cooled and solidified with air.[6] Heat-sensitive drugs, such as proteins, cannot be used in this spinning method because they are destroyed during the spinning.

To overcome this problem the melt-spun polylactide fibers can be coated with the heat-sensitive drug, and thus the drug is not exposed to high temperature. Levy and Zilberman[7] have prepared core/sheath composite fiber structures. The core was a melt-spun P(L)LA fiber and the sheath was coated with an emulsion containing poly(DL-lactide-*co*-glycolide) dissolved in chloroform and protein dissolved in water. The diameter of this composite fiber was about 200 μm.

Another way to overcome the problem is wet-spinning. It can be conducted at room temperature and the heat-sensitive drugs can be incorporated inside the fiber, not just on the fiber surface. In the wet-spinning process the polymer is dissolved in solvent; the polymer solution is pumped through a spinneret into a spin bath, where the polymer is precipitated and the filaments are reeled. The spin bath includes polymer nonsolvent and possible additives.[6] Wet-spinning of enantiomerically pure polylactide has already been introduced several years ago.[8,9] In our previous study we presented the wet-spinning of polylactide stereo copolymer multifilament fibers.[10]

Gao et al.[11] have fabricated a 5-fluorouracil-loaded P(L)LA fiber using the wet-spinning method. They have prepared a suspension from P(L)LA dissolved in chloroform and drug powder, and extruded it into a methanol/isopropanol spin bath. The drug particle size was 0.5–5 μm and the diameter of the fiber varied from 50 to 250 μm.

Crow and Nelson[12] have fabricated protein-loaded core/sheath fibers using coextrusion wet-spinning technology. The core was a hydrogel prepared from sodium alginate and protein, and the sheath was made from P(L)LA. Polymer was dissolved in chloroform/iso-ocatane 65/35 mixture, and the polymer and hydrogel were extruded by a syringe into a pentane spin bath. The fibers were rather thick, with diameters in the range of 200–500 μm. Polacco et al.[13] have fabricated hollow fibers containing drug-loaded nanoparticles. Poly(DL-lactide-*co*-ε-caprolactone) was dissolved in acetone and it was coextruded with a nanoparticle-water system into a water spin bath.

A novel approach to fabricating heat sensitive drug-loaded fibers is an electrospinning method; in this method polymer solution is extruded through a nozzle and polymer jets are attracted to the collector electrode using electrostatic forces. This spinning method is used for nano-sized diameter fiber fabrication. Qi et al.[14] have fabricated protein-loaded nanofibres by the emulsion electrospinning method. P(L)LA was added to the emulsion containing Ca-alginate, protein, water, and chloroform. Ca-alginate-protein was shown as microbeads in the fibers and the diameter was about 7 μm.

The aim of this research was to study the properties of protein-loaded multifilament fibers fabricated by wet-spinning. Bovine serum albumin (BSA) was used as a model drug to simulate the heat sensitive drug. The previous studies of drug-loaded fiber fabrication have been focused on electrospinning or wet-spinning of monofilament by a one-hole nozzle and a syringe pump. In this study we fabricated wet-spun multifilaments using an industrial-scale multihole nozzle and a gear pump. The multihole nozzle enables the manufacturing of finer filaments compared to the one-hole nozzle. Also the production capacity of multihole nozzle is bigger than that of the one-hole nozzle which enables faster fiber production, for example to the nonwoven manufacturing. As fiber polymers were used polylactide stereo copolymers which are more amorphous than enantiomerically pure polylactide.

EXPERIMENTAL

Materials

Two medical grade stereocopolymers were purchased from Purac Biochem BV (Gorinchem, The Netherlands). The polymers were poly(L,D-lactide), [P(L,D)LA], L/D ratio 96/4 and P(L,DL)LA, L/DL ratio 70/30. The intrinsic viscosities (η) were given by the supplier, the viscosity average molecular weights (M_v) were determined by the gel permeation chromatography,[15] and the degrees of crystallinities (X) were determined by the differential scanning calorimeter.[10] They are shown in Table I. BSA, minimum 96% electrophoresis, was purchased from Sigma-Aldrich Co. (St. Louis).

Spin dope preparation and wet-spinning of protein-loaded filaments

The polymer was dissolved in dichloromethane (analytical grade) in a conical flask covered by a glass

TABLE I
Tested Copolymers, their Intrinsic Viscosities (η), Molecular Weights (M_v), Degrees of Crystallinities (X), and Spin Dope Concentrations

Polymer	η (dL/g)	M_v (g/mol)	X (%)	Spin dope concentration (%)
P(L,D)LA 96/4	2.2	93 700	40	10
P(L,DL)LA 70/30	3.1	167 200	14	8

stopper at room temperature. The magnetic stirrer was used to mix solvent and polymer until the solution was clear. The spin dope concentrations were calculated from the volume of solvent and they are given in Table I.

BSA was dissolved in distilled water; the amount of BSA was 2% of the weight of polymer and the volume of water was 6% of the volume of dichloromethane. The polymer solution and the protein solution were probe sonicated (Dr. Hielscher UP200S, Teltow, Germany) at 200 W (100% amplitude) for a total time of 9 min using 0.7–0.3-s on-off duty cycle. A cooled (5°C) metal container was used to limit the heating of the mixture. The probe sonicator was used to mix the polymer solution and water solution because it generated small protein-water dispersion into the polymer solution. The protein solution had to be very finely dispersed to minimize the negative effect of water dispersion during the filament spinning.

The protein-loaded filaments were manufactured using the wet-spinning method. The polymer-protein solution was transferred to the tank, and it was pumped using the Zenith gear pump (Allweiler GmbH, Radofzell, Germany) through the spinneret (10 holes, hole diameter 0.15 mm; Enka Technica GmbH, Heinsberg, Germany) to the ethanol (analytical grade) containing coagulation bath as shown in Figure 1. The coagulated filaments were reeled to the bobbin (diameter 87 mm). The utilized feed rate was 1.1 mL/min, the spinneret velocity was 7 m/min, the reeling velocity was 9 m/min, and the calculated spin draw ratio was about 1.3 (the reeling velocity divided by the spinneret velocity). The calculated coagulation time of the filaments was 6 s.

The filaments were evacuated in a vacuum oven at 37°C overnight to eliminate chemical residues. They were stored in a desiccator at room temperature until their testing to avoid the moisture intake. The filling of the desiccator was dried silica gel.

Characterization of filaments

A scanning electron microscope (SEM) (Jeol JSM-T100, Jeol, Tokyo, Japan) was used to characterize the filament surface and cross-section. A projection microscope (Projectina, Projectina AG, Heerbrugg, Swizerland) was used to determine the filament diameter. The mean of diameter was calculated from 50 individual filaments.

The breaking force and elongation at break were tested from 50 individual filaments using the tensile testing machine (Vibrodyn by Lenzing AG, Lenzing, Austria). The gauge length was 20 mm, the testing speed was 20 mm/min, and the maximum force of the load cell was 100 cN. Because the diameters of the filaments differed from each other the breaking force was converted to the tenacity.

For the *in vitro* protein release study three parallel filament bundles (500 mg) were placed in test tubes and the tubes were filled (5 mL) with soaking solution (phosphate buffer solution).[16] The filled test tubes were kept at a constant temperature 37°C ± 1°C. Samples of 5 mL solution were collected periodically and their BSA contents were determined via a standard curve by measuring absorbance at 279.0 nm, with the use of a Unicam UV 540 spectrometer (Thermo Spectronic, Cambridge, UK). The data points were 1, 2, 3, 5, 9, 13, 17, 21, and 24 weeks. A

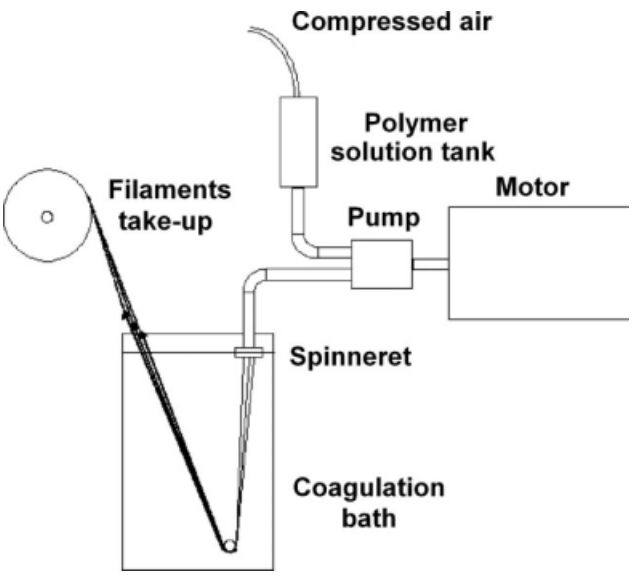

Figure 1 Schematic drawing of wet-spinning equipment.

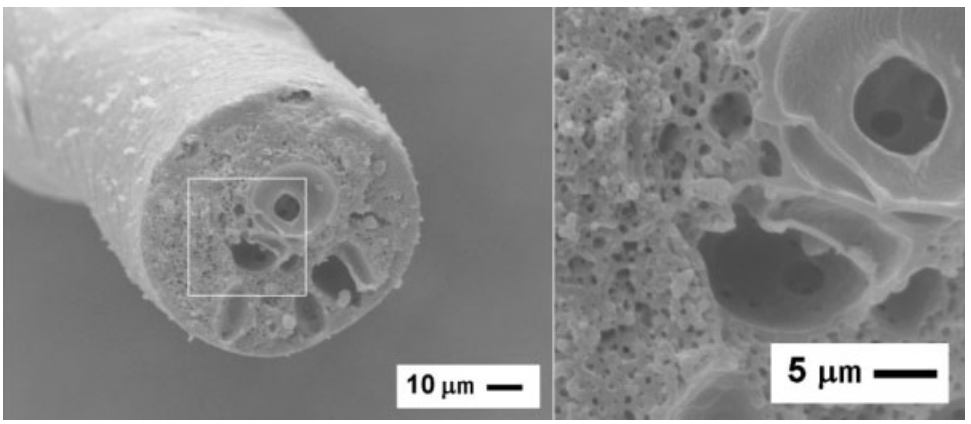

Figure 2 SEM-images of protein-loaded P(L,D)LA 96/4 filament.

fresh soaking solution of 5 mL was transferred to the test tubes by a pipette.

RESULTS AND DISCUSSION

Wet-spinning of protein-loaded multifilaments

The preparation of the polymer-protein emulsion increased the viscosity of the spin dope, and thus the lower polymer concentration was utilized. The spin dope concentration was 10% for P(L,D)LA 96/4 and 8% for P(L,DL)LA 70/30. Respectively, the spin dope concentrations have been 15% and 10% for the unloaded filaments.[10] The sufficient large hole diameter of the spinneret ensured spinning with the minimal number of filament breakages. In this study the hole diameter of the spinneret was 0.15 mm, whereas for the unloaded filaments the 0.1 mm hole diameter was utilized. Despite of the larger hole diameter, the fabrication of protein-loaded wet-spun filament was more difficult than the spinning of unloaded filaments. The polymer emulsion contained very small air bubbles which caused difficulties in the filament spinning. For example, the reeling velocity was as low as 9 m/min in the present study, whereas it was as high as 70 m/min for the unloaded filament.

Surface and inner structure

The surfaces of both stereo copolymer filaments were smooth. It can be observed the large and small pores in the cross-sections of filaments (Figs. 2 and 3). The sonication of the protein-polymer solution formed air bubbles to the spin dope, and thus the large pores could be the air bubbles in the spin dope. They could also be generated due to the serious phase separation of the emulsion. Both filaments also contained a high number of small pores which were formed during the coagulation of the filaments. The formation of a structure with small pores is typical for wet-spun filaments. When the filament was immersed into the spin bath the filament skin was solidified immediately, and solvent and nonsolvent were trapped inside the filament. The small pores were formed when solvent and nonsolvent were evaporated during the drying.[17] A similar porous structure has also been observed in other wet-spinning studies.[9,11,18]

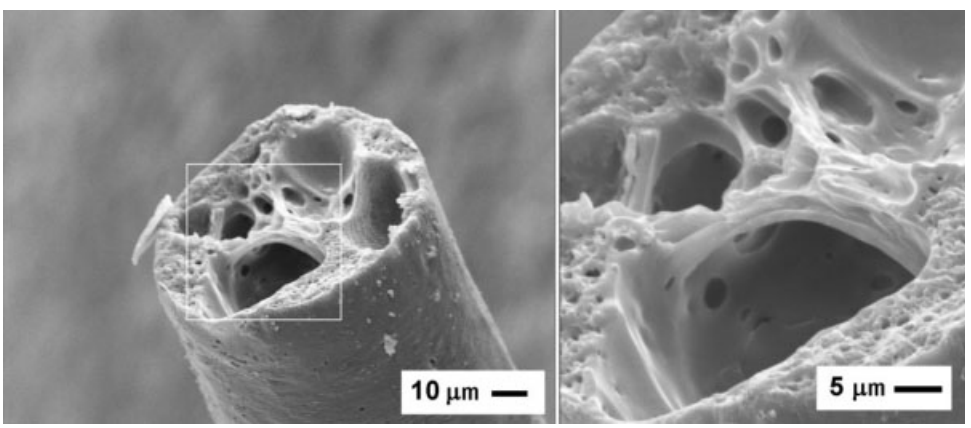

Figure 3 SEM-images of protein-loaded P(L,DL)LA 70/30 filament.

TABLE II
Diameters, Tenacities, Young's Modulus Values, and Elongation at Break Values of Protein-Loaded Filaments

Filament	Diameter (μm)	Tenacity (MPa)	Young's modulus (GPa)	Elongation at break (%)
P(L,D)LA 96/4	46 ± 13	16.5 ± 3.3	0.51 ± 0.14	74 ± 32
P(L,DL)LA 70/30	70 ± 19	7.1 ± 1.9	0.36 ± 0.05	62 ± 41

Mechanical properties

The filament diameters are presented in Table II. The mean diameter of P(L,D)LA 96/4 was 46 μm and that of P(L,DL)LA 70/30 was 70 μm. The P(L,DL)LA 70/30 filaments were thicker than the P(L,D)LA 96/4 filaments, and this might have been caused by the larger air bubbles inside the P(L,DL)LA 70/30 filaments. However, our filaments fabricated by multinozzle spinneret were as thin as or thinner than the filaments which were fabricated by the syringe in the other studies.[11,12]

The mechanical properties of the protein-loaded filaments are also presented in Table II. The tenacity of the P(L,D)LA 96/4 filament was 16.5 MPa and that of P(L,DL)LA 70/30 was only 7.1 MPa. The Young's modulus values were 0.51 GPa for P(L,D)LA 96/4 and 0.36 GPa for P(L,DL)LA 70/30. The mechanical properties of the protein-loaded filaments were much lower than that of the unloaded wet-spun filaments made from similar copolymers and spinning parameters.[10] The mechanical strength values of the protein-loaded and the unloaded filaments are not fully comparable because the spin dope concentrations were different. The decrease in the spin dope concentration decreases the filament properties.[19] The lower spin dope concentrations of the protein-loaded filaments do not explain all the reduction of the mechanical properties. The low mechanical properties might have been caused by a high number of pores inside the filaments.

The stress–strain curves of the protein-loaded filaments are presented in Figure 4. In the beginning of the curves there was a linear Hookean region. In this region the molecular chains started to stretch and the molecules became straight in the amorphous region of the filament, and also the intermolecular bonds stretched. The yield stress of the P(L,DL)LA 70/30 filament was lower than that of the P(L,D)LA 96/4 filament which was probably caused by the higher porosity of the P(L,DL)LA 70/30 filament. After the Hookean region came a region of easier extension, where the highly stretched bonds in the amorphous region could not withstand the force which resulted in them breaking. The extension became easier because the molecules became further straightened, and the load of the other bonds increased. The increasing stretch affected on the bonds and molecules, and finally the filament broke.[20]

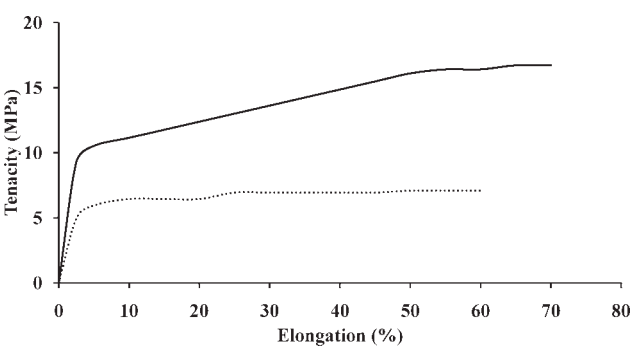

Figure 4 Stress–strain curves of protein-loaded fibers: (-) P(L,D)LA 96/4 and (..) P(L,DL)LA 70/30.

In vitro degradation

Polylactide is degradaded by hydrolysis in the human body and the soluble oligomers are metabolized by cells. During hydrolysis water initally diffuses into the amorphous regions of the polymer and causes breakage of the ester bonds which initiates a reduction in molecular weight and then later, a reduction in mechanical strength. After the amorphous regions hydrolysis occurs in the crystalline regions leading to increased mass loss and finally to complete resorption.[21]

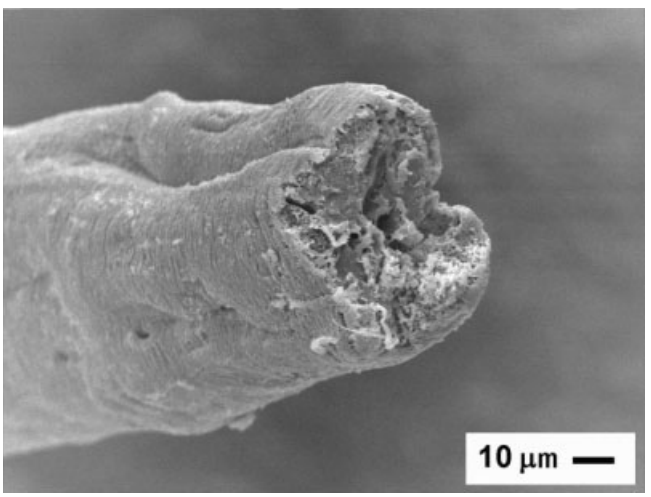

Figure 5 SEM-image of protein-loaded P(L,D)LA 96/4 filament after 24 weeks *in vitro*.

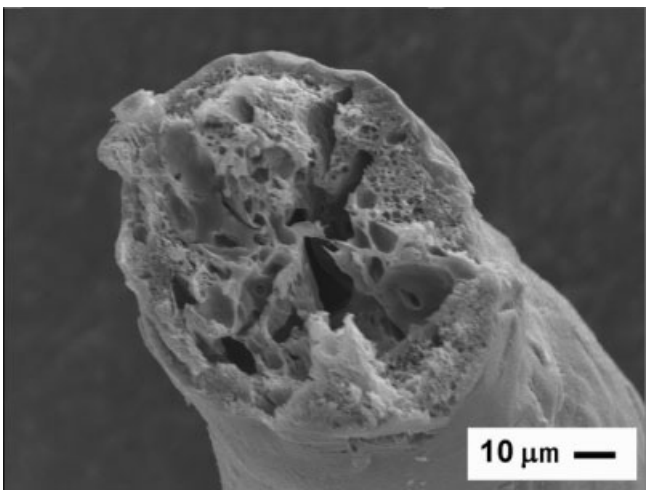

Figure 6 SEM-image of protein-loaded P(L,DL)LA 70/30 fibers after 24 weeks *in vitro*.

The SEM-images of the protein-loaded filaments after 24 weeks *in vitro* are presented in Figures 5 and 6. After this period it was observed that distinct erosion occurred in the cross-section of the P(L,DL)LA 70/30 filament as shown in Figure 6. The erosion was not so clear with the P(L,D)LA 96/4 filament (Fig. 5). Li et al.[22] have noticed a more rapid degradation in the centre of the amorphous specimen than at the surface. They have suggested that the amorphous polylactide specimen has absorbed the aqueous medium and the breakages of ester bonds have started from the centre, and the specimen have become hollow gradually. The acid degradation products of polylactide can cause autocatalytic effects leading to faster erosion inside compared to the surface.[23] On the other hand Nishimura et al.[24] have observed a regular pattern of cracks along the vertical direction of the semicrystalline P(L)LA filament. Gupta et al.[25] have observed similar surface deterioration.

In this study the mechanical properties were not measured during *in vitro* testing. However, after 16 weeks *in vitro* the mechanical strength of the protein-loaded P(L,DL)LA 70/30 filaments was so low

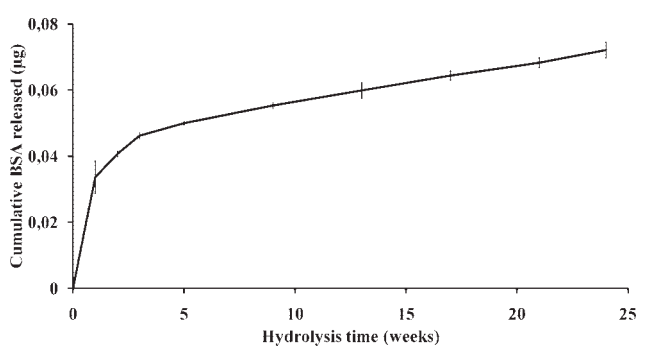

Figure 7 *In vitro* cumulative BSA released from the protein-loaded P(L,D)LA 96/4 filament.

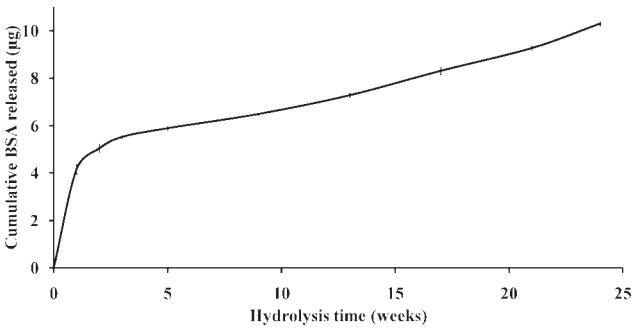

Figure 8 *In vitro* cumulative BSA released from the protein-loaded P(L,DL)LA 70/30 filament.

that the filaments started to break and there were short filaments in the test tube. In our previous study it was observed that the degradation rate of the P(L,DL)LA 70/30 filament has been faster than that of P(L,D)LA 96/4.[10]

The cumulative BSA release curve of the protein-loaded P(L,D)LA 96/4 filament is presented in Figure 7 and that of the protein-loaded P(L,DL)LA 70/30 filament in Figure 8. It can be observed that there was a burst effect in the beginning of both curves. During this period protein molecules from the surface were released into the soaking solution. After this period the release was slower because the soaking solution had not yet affected the filament polymer. The BSA release was slightly accelerated with the P(L,DL)LA 70/30 filament after 9 weeks as Veiranto et al.[4] have observed. The acceleration of the BSA release was not observed with the P(L,D)LA 96/4 filament. Also the total amount of the released BSA was lower with the P(L,D)LA 94/6 filament.

The difference in the release rates of filaments is due to the different degradation times of filaments. P(L,DL)LA 70/30 was more amorphous, and its degradation time was shorter than that of P(L,D)LA 96/4.[10] In addition, the drug release rate is determined by the diffusion of the drug molecule.[26] BSA is a single polypeptide chain, and its molecular weight is about 66,500 according to the supplier. It is a very big molecule, and thus the diffusion of BSA from the polymer matrix is negligible.

The possible conformation change of BSA due to the denaturation effect of the spin bath coagulant was not evaluated in this study. However, the possible conformation change was tried to minimize by using as short coagulation time as possible during the spinning process.

CONCLUSIONS

It was possible to add the heat-sensitive drug, as protein, to a polymer solution and fabricate continuous multifilaments by the wet-spinning method. The

water-soluble protein was mixed with the polymer solution using a probe sonicator to form a finely dispersed emulsion. The mechanical strength values of both protein-loaded filaments were very low, and the strength is not sufficient for the end-uses where they would be required to withstand the load. For example, in the fabrication of the nonwoven, it is advisable to blend protein-loaded fibers with unloaded fibers to achieve better mechanical properties. The release rate of the protein was low with both polymers due to the low diffusion of the protein and the slow degradation rate of the polymer. If a higher release rate of protein is needed, the use of a different type of polymer is recommended.

References

1. Harrison, K. Biomedical Polymers; Jenkins, M., Ed.; Woodhead Publishing: Cambridge, 2007; Chapter 2.
2. Bergsma, J. E.; De Bruijn, W. C.; Rozema, F. R.; Bos, R. R. M.; Boering, G. Biomaterials 1995, 16, 267.
3. Kellomäki, M.; Pohjonen, T.; Törmälä, P. Biodegradable Polymers; Arshady, R., Ed.; Citus: London, 2003; Chapter 7.
4. Veiranto, M.; Törmälä, P.; Suokas, E. J Mater Sci Mater Med 2002, 13, 1259.
5. Linneman, B.; Sri Harwoko, M.; Gries, T. Chem Fibers Int 2003, 53, 426.
6. Fourne, F. Synthetic Fibers—Machines and Equipment Manufacture, Properties; Hanser: Munich, 1999.
7. Levy, Y.; Zilberman, M. J Biomed Mater Res 2006, 79, 779.
8. Kulkarni, R. K.; Pani, K. C.; Neumann, C.; Leonard, F. Arch Surg 1966, 93, 839.
9. Nelson, K. D.; Romero, A.; Waggoner, P.; Crow, B.; Bornemann, A.; Smith, G. M. Tissue Eng 2003, 9, 1323.
10. Rissanen, M.; Puolakka, A.; Hukka, T.; Ellä, V.; Nousiainen, P.; Kellomäki, M. J Appl Polym Sci 2009, 113, 2683.
11. Gao, H.; Gu, Y.; Ping, Q. J Controlled Release 2007, 118, 325.
12. Crow, B. B.; Nelson, K. D. Biopolymers 2006, 81, 419.
13. Polacco, G.; Cascone, M. G.; Lazzeri, L.; Ferrara, S.; Giusti, P. Polym Int 2002, 51, 1464.
14. Qi, H.; Hu, P.; Xu, J.; Wang., A. Biomacromolecules 2006, 7, 2327.
15. Ellä, V.; Kellomäki, M.; Törmälä, P. J Mater Sci Mater Med 2005, 16, 655.
16. Shah, S. S.; Chay, Y.; Pitt, C. G. J Controlled Release 1992, 18, 261.
17. Frushour, B. G.; Knorr, R. S. Handbook of Fiber Chemistry; Lewin, M., Ed.; CRC: Boca Raton, FL, 2007; Chapter 12.
18. Gupta, B.; Revagade, N.; Anjum, N.; Atthoff, B.; Hilborn, J. J Appl Polym Sci 2006, 100, 1239.
19. Walczak, Z. Processes of Fiber Formation; Elsevier: Oxford, 2002.
20. Morton, W. E.; Hearle, J. W. S. Physical Properties of Textile Fibers; Textile Institute: Manchester, 1997.
21. Vert, M.; Li, S. M.; Spenlehauer, G.; Guerin, P. J Mater Sci Mater Med 1992, 3, 432.
22. Li, S. M.; Garreau, H.; Vert, M. J Mater Sci Mater Med 1990, 1, 123.
23. Göpferich, A. Handbook of Biodegradable Polymers; Domb, A. J.; Kost, J.; Wiseman, D. M.; Eds. CRC: Boca Raton, FL, 1997; Chapter 22.
24. Nishimura, Y.; Takasu, A.; Inai, Y.; Hirabayashi, T. J Appl Polym Sci 2005, 97, 2118.
25. Gupta, B.; Revagade, N.; Hilborn, J. J Appl Polym Sci 2007, 103, 2006.
26. Kwon, G. S.; Furgeson, D. Y. Biomedical Polymers; Jenkins, M., Ed.; Woodhead Publishing: Cambridge, 2007; Chapter 4.