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Technical Report

Technique Paper for Wet-Spinning Poly(L-lactic acid) and Poly(DL-lactide-co-glycolide) Monofilament Fibers

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ABSTRACT

A simple and repeatable method is described for wet-spinning poly(L-lactic acid) (PLLA) and poly(DL-lactic-co-glycolic acid) (PLGA) monofilament fibers. These fibers are strong, elastic, and suitable for many applications, including use as tissue-engineering scaffolds. The PLLA wet-extruded fibers do not show additional strain-induced crystallization as a result of drawing the fibers during fabrication; however, there is an apparent increase in crystallinity late in the degradation process in saline at 37°C. We have measured the molecular weight degradation in saline at 37°C for fibers of both PLLA and PLGA. Changing solvent systems, polymer blends, and winding rates alters mechanical and morphological properties of these fibers for specific applications. The authors discuss a possible theoretical explanation for these observed changes due to changes in polymer concentration, solvent system, and coagulation bath properties. This wet-extrusion process is simple and inexpensive enough to be carried out in almost any laboratory interested in tissue engineering.

INTRODUCTION

POLY(L-LACTIC ACID) (PLLA) poly(glycolic acid) (PGA), and their copolymers and blends have been used as bioresorbable polymers in medical applications since the 1960s.[1] They have been used as dissolvable sutures,[2] in orthopedic applications,[3,4] and more recently have become important synthetic scaffoldings for tissue-engineering applications.[5–11] They were chosen because they have FDA approval in many applications, they were found to have good strength, were readily processed, and easy to obtain, purify, and use in bulk quantities.

The fiber format was nearly always obtained by conventional melt-extrusion techniques; however, because of the size and cost of melt-extrusion equipment, and the large amount of raw material required, it has not been well suited to bench-top, laboratory quantities. Therefore, we sought other processing methods to obtain similar fibers. This article describes simple, inexpensive, bench-top techniques for wet-spinning PLLA and poly(DL-lactide-co-glycolide) (PLGA) monofilament fibers suitable for scaffoldings for tissue-engineering applications. The concept of wet-spinning is not new; Kulkarni *et al.* wet-spun PLLA fibers as far back as 1966.[1] However, wet-spinning has generally produced fibers not as mechanically strong as fibers produced by melt-extruding, and therefore has not been investigated as thoroughly. Therefore, we felt that a technical report to teach the concepts

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and techniques of wet-spinning would be of great benefit to the majority of tissue engineers who are currently using PLLA or PLGA fibers in their research. The techniques described herein avoid the large capital, space, and raw material requirements of conventional melt-extrusion of these polymers; and further, they demonstrate that fiber properties are controllable and tunable for specific applications, thus making this technique more versatile than melt-extruding. For example, wet-spinning into PEG 600 creates an interpenetrating network of PEG with the PLLA, which results in a highly hydrophilic fiber, although with good mechanical properties; which would be impossible to obtain by conventional melt-extrusion techniques. This technique may also lend itself to the incorporation of heat-sensitive drugs into the fiber, as the entire process takes place at room temperature.

We have been using these techniques successfully for approximately 6 years to culture a number of cell types, such as human fetal foreskin fibroblasts, rabbit corneal fibroblasts, human umbilical vein endothelial cells, rat fibroblasts, mouse immature myoblasts, and rat pup dorsal root ganglion cells *in vitro*. We have also used these fibers *in vivo* to create a peripheral nerve graft, which was capable of successfully inducing axonal elongation up to 18 mm in a rat sciatic nerve resection model.[12,13] These fibers have caused no apparent problems *in vitro* when used without further modification. *In vivo* unmodified fibers have evoked a mild to moderate inflammatory response in rat spinal cord and rat sciatic nerve implantation, but not with intraocular implantation. We have also used simple dip-coating techniques of laminin[14] and collagen to improve cellular adhesion *in vitro*.

MATERIALS AND METHODS

Fiber fabrication by a wet-spinning technique

Poly(L-lactic acid) (50–200 kDa; Polysciences, Warrington, PA) was dissolved in varying concentrations from 7.5 to 15 wt/v% in a variety of solvent systems, including chloroform (Aldrich Chemical, St. Louis, MO), 1,4-dioxane, a 5:1 mixture of chloroform–toluene, and a 4:1 mixture of chloroform–hexane.

PLGA (intrinsic viscosity, 0.66–0.80; Polysciences) was dissolved in methylene chloride or chloroform at a concentration of 20 wt/v%. We allowed a minimum of 90 min for complete dissolution of all polymer solutions.

The polymer solution was loaded into a glass syringe (gas-tight syringes; Hamilton, Reno, NV) and placed in a syringe pump (model 945 [Harvard Apparatus, Holliston, MA] or model KDS200 [KD Scientific, New Hope, PA]). The polymer flow rate was typically between 0.02 and 0.1 mL/min. A Viton tube (Cole-Parmer, Vernon Hills, IL) connected the syringe to the needle dispensers. We used only blunt-tipped needles (Small Parts, Miami Lakes, FL), because the bevel cut on sharp needles would cause problems during extrusion. We immersed the tip of the needle in a coagulation bath, which was a poor solvent for the polymer, and yet was highly miscible with the solvent used to dissolve the polymer. The coagulating baths we have used include isopropyl alcohol, short alkanes (heptane, cyclohexane, hexane, and pentane) and poly(ethylene glycol) (PEG) 200 and 600 M_w, and amphiphilic polymers including several of the Pluronics family (BASF). The fiber mechanical and physical properties such as strength, diameter, surface tension, and so on are strongly dependent on the choice of coagulation bath. If the precipitation of the polymer fiber was too rapid, up to 20 v/v% of the polymer solvent may have been added to the coagulation bath.

The original extrusion setup was a narrow aluminum tank with overall dimensions of approximately 60 × 30 × 3 cm with two movable rollers. Lowering the rollers into the tank after the fiber began to form provided a fixed path length for the fiber.[15] This setup was used to generate much of the data in this article; however, we have since simplified this setup to a simple glass tube (i.e., 25 mm i.d., length, 20 cm) with a rubber septum at the top through which the dispensing needle was pierced. The glass tube was immersed in a small container below, as shown in Fig. 1, and filled with the coagulation bath fluid. When the fiber exited the coagulating bath (from either setup) it was wound on a 8.25-cm-diameter bobbin attached to the jaws of a modified 5-in. garden lathe (Sears-Craftsman model 549-289000; Sears and Roebuck, Chicago, IL). A 24-V power supply (model 5005R; Power Designs, NY) drove a 0.03-horsepower DC motor (Pittman Motors, Harleysville, PA) that had replaced the original motor on the lathe. We first wrapped the bobbin with paper so that on completion of the extrusion run, we would pull the paper from the bobbin to remove the fiber intact. The angular velocity of the lathe was measured with an optical tachometer. The draw ratio was calculated as the ratio of the linear velocity of the fiber mea-

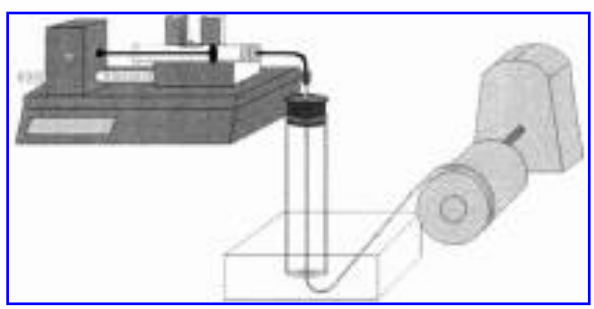

FIG. 1. Schematic diagram of wet extrusion set-up, showing a syringe pump with a mounted syringe to extrude the polymer into a glass cylinder filled with coagulation fluid. The newly formed fiber is wound on a bobbin attached to a modified lathe.

sured at the take-up bobbin to the calculated mean linear velocity of the polymer solution within the dispensing needle. We typically observed no die-swell during these extrusions; therefore, we used the mean linear velocity within the dispensing needle for draw ratio calculations.[16] These techniques readily produced draw ratios as high as 40:1.

At the end of the extrusion run, fibers were removed from the lathe, left on the paper roll, and placed under vacuum at room temperature for at least 4 h at room temperature to help remove remaining solvents. The fiber was stored in a desiccator or in a −20°C freezer until needed.

To generate fibers that were not wound, it was possible to simply extrude into an Erlenmeyer flask or even a 50-mL conical Falcon tube that was filled with the coagulation fluid. At the end of extrusion, these fibers were collected, placed under vacuum, and stored as described above.

Tensile test apparatus

A motorized platform was constructed to fit into a cabinet, on top of which an electric balance (model AE240; Mettler Toledo, Worthington, OH) was placed. The fiber was tied to a metal fixture approximately 1 mm in diameter, which was secured to the underscale attachment on the electronic balance through a hole in the top of the cabinet. Similarly, the other end of the fiber was tied around a similar holder, which was attached to the movable platform. The initial, unstressed fiber length was 5 cm. After taring the balance, the platform descended at a steady rate of 2.78 mm/min. We converted the electronic balance measurements of the induced load along with the position of the platform into stress strain data. If the fiber failure occurred at the knot, or if any slipping of the knot occurred, the data were discarded. Ultimate stress and strain were calculated for each fiber sample, using the initial diameter of the fiber (obtained from scanning electron micrographs taken before any mechanical testing). Our experience has shown that the diameter was relatively constant over the length of the fiber.

PLLA sample preparation for DSC measurements

We calculated percent crystallinity on the basis of thermograms produced by differential scanning calorimetry (model 2010; TA Instruments). The samples were vacuum dried at 70°C for 2 h and then weighed, pressed to form a disc, and placed inside aluminum pans. The reference was an empty aluminum pan. The temperature profile runs from 30 to 250°C at a constant rate of 10°C/min under a nitrogen purge. Integration of the melting and crystallization peaks produced the heat of fusion (ΔH_f) and heat of crystallization (ΔH_c) for each sample in joules per gram. We calculated percent crystallinity by

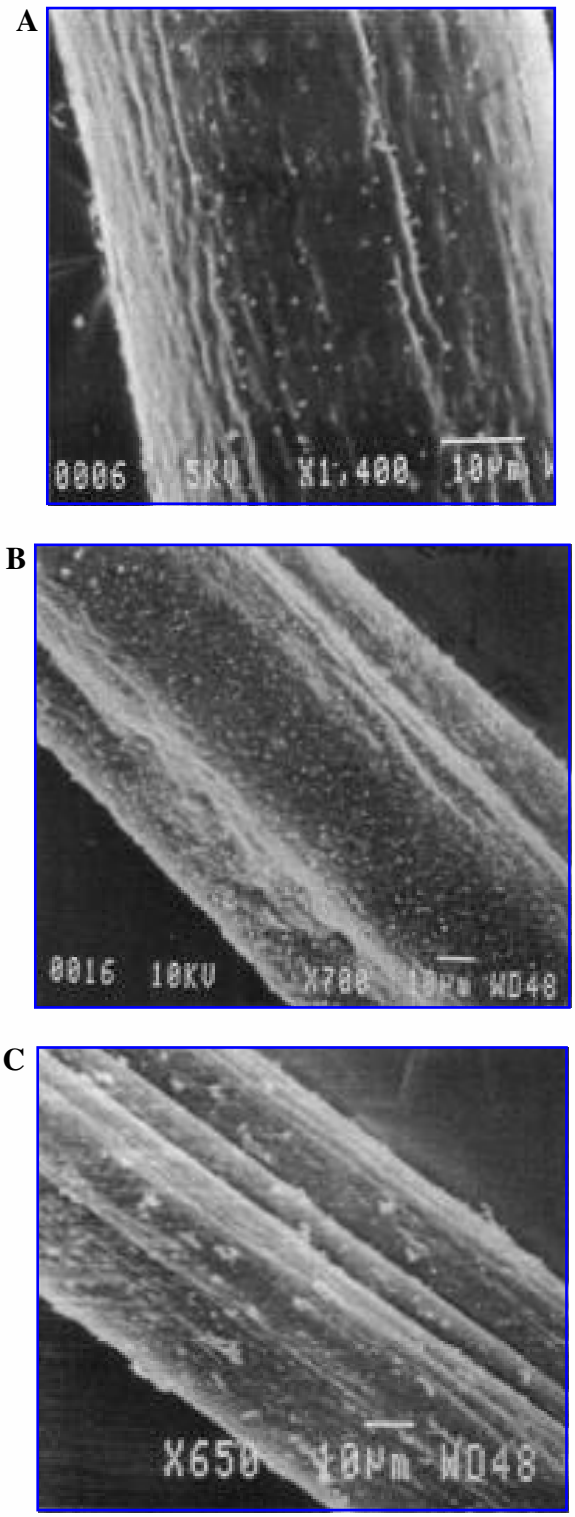

FIG. 2. Scanning electron micrographs of various fibers. Each fiber in (**A**–**C**) was extruded through an 18-gauge needle into isopropyl alcohol. (**A**) PLLA (10 wt/v%) in chloroform; (**B**) PLLA (8 wt/v%) in chloroform; (**C**) PLLA (7.5 wt/v%) in chloroform.

subtracting ΔH_c from ΔH_f and dividing by 93.6 J/g, which is ΔH_f for a pure PLLA crystal.[17]

Molecular weight degradation studies

The fibers were cut into pieces (typically 33 cm), carefully weighed, dipped in 70% ethanol for 15 s to minimize bacterial contamination, placed in sterile Eppendorf tubes, and covered with 1.0 mL of phosphate-buffered saline (pH 7.4) with 0.01 wt%. Thimerasol as a broad-spectrum antibiotic and incubated at 37°C. At weekly intervals, the spent PBS was replaced with fresh PBS in each sample, and three fibers were removed from the PBS, dried with a Kimwipe, and stored in the freezer (−20°C) until analyzed. The pH of the spent medium was measured. The molecular weight was determined by dissolving each sample in 3.0 mL of methylene chloride and injecting it into an HPLC (Akta purifier 10; Amersham Biosciences, Piscataway, NJ) and analyzing using UV at 236 nm and RI detectors (Shodex R-71; Showa Denko, Tokyo, Japan). We used a GPC column (Asahipak GPC guard column model GF-1G7B [Phenomenex] and TSK gel column model G2000-HHR [Supelco]) (10-μL injection loop) with methylene chloride as the mobile phase at flow rate of 1.0 mL/min. M_n and M_w were calculated from the chromatograms on the basis of polystyrene molecular weight standards (Supelco).

RESULTS

Physical and mechanical characterization

Over the past 6 years, hundreds of fibers have been produced with a wide range of physical and mechanical properties; for example, we have produced fibers with diameters as small as 28 μm, and as large as 550 μm, only a small fraction of which are reported here. The chosen solvent system and polymer composition greatly affected the external morphology of the filaments as shown by scanning electron microscopy (SEM) of various PLLA fibers (Fig. 2). We also noted morphological changes simply by varying the concentration of the polymer solution under otherwise identical extrusion conditions (Fig. 2). In all these cases, the dispensing tip was an 18-gauge needle and the coagulating bath was isopropyl alcohol. These different surface morphologies and textures may be advantageous for tissue-engineering applications. For example, longitudinal grooves may provide contact guidance and increased surface area for cell attachment.

Tensile strength may determine the usefulness of the filaments in a variety of applications. Early on, we discovered that the ultimate tensile strength was an interesting function of draw ratio for different initial polymer concentrations (Fig. 3). Dramatic changes in tensile strength occur over relatively small changes in the draw ratio from 23:1 to 26:1, but after that it remains relatively constant out to a draw ratio of 40:1. It is interesting to

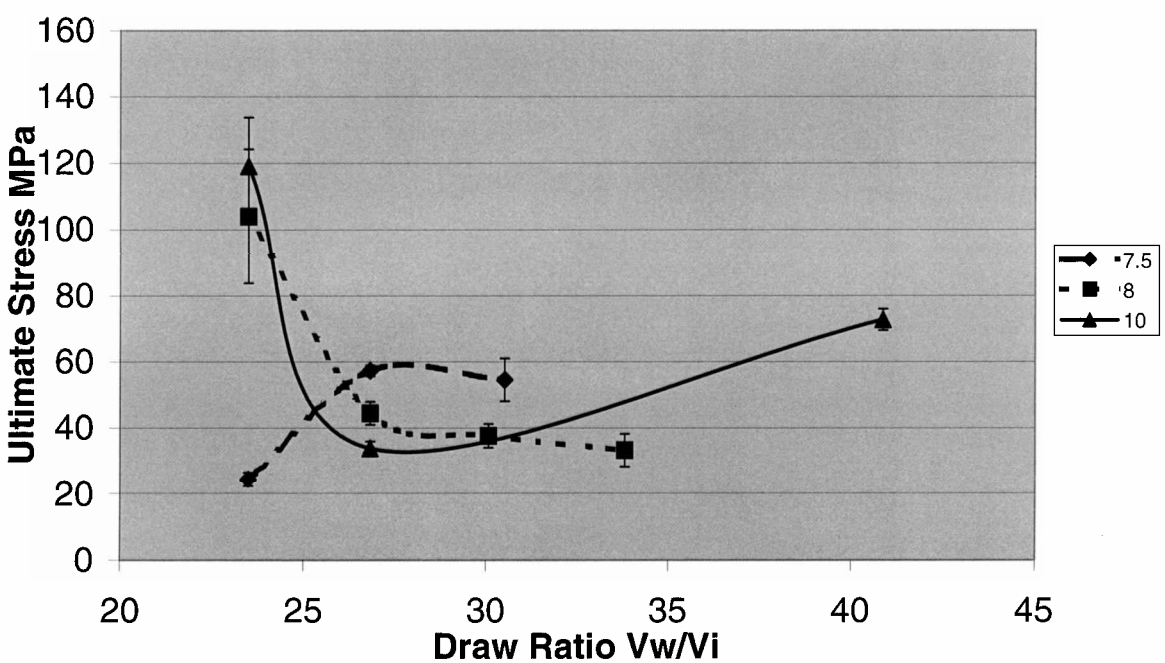

FIG. 3. Ultimate tensile stress as a function of winding ratio V_w/V_i; where V_w is the winding velocity at the bobbin and V_i is the mean linear velocity within the dispensing tip. Shown are data for fibers made from PLLA (7.5, 8, and 10 wt/v% in chloroform) extruded through an 18-gauge needle into isopropyl alcohol.

compare the similarity in behavior of the 8 and 10 wt/v% polymer concentration fibers, and to contrast it with the behavior of 7.5 wt/v% polymer concentration fibers; the 8 and 10 wt/v% polymer concentration fibers showed a counterintuitive precipitous drop in ultimate tensile stress as the draw ratio increased from 23:1 to 26:1. This drop in ultimate tensile stress occurred despite a decrease in cross-sectional area, thus indicating that there was a substantial decrease in the load-carrying ability in these fibers. For the 7.5 wt/v% concentration fibers, however, an increase in tensile stress was observed over these same draw ratios. Unfortunately, high draw ratios are more difficult to achieve as the polymer concentration of the spinning solution decreases, which made comparison at higher draw ratios impossible across all polymer concentrations. These tensile strengths are for PLLA fibers dissolved in chloroform, and extruded into isopropyl alcohol through an 18-gauge dispensing tip.

Molecular weight and crystalline characterization

The measured pH of the spent medium for PLLA fibers did not drop below pH 6.8 for any given week, but for PLGA fibers the pH dropped to pH 4.8. Therefore, acidic autocatalysis may play a role in the measured degradation rate of the PLGA fibers. The PLGA (50:50) (initial i.v., 0.66–0.8) degraded in approximately 2 months,[18] which was also verified in our *in vitro* molecular weight degradation studies (Fig. 4).

Polymers made from the L-isomer of poly(lactic acid) are inherently crystalline, as are polymers made from nonchiral but chemically related poly(glycolic acid), whereas polymers made from the racemic DL isomer mixture are amorphous, as are copolymers of glycolic and lactic acids.[19] Percent crystallinity was therefore an important qualitative parameter for mechanical strength and degradation rate determination for PLLA fibers.

Table 1 lists fiber diameter as determined from SEM (not shown), density calculated from the mass of a given length of fiber assuming that the diameter remains constant, and percent crystallinity calculated by integrating the peaks of the thermograms as explained above. These fibers were all made from 10 wt/v% PLLA in chloroform at various draw ratios with a 21-gauge needle. Diameter linearly decreased with draw ratio, and density linearly increased with draw ratio except for the 26:1 draw ratio, which fell outside the expected linear range for both density and percent crystallinity. Percent crystallinity does not appear to be a function of draw ratio, nor does it change substantially as a function of time during 37°C saline incubation (Fig. 5). This will be an unexpected finding for those familiar with melt-extrusion, where strain-induced crystallization generally results in increased crystallinity as the draw ratio increases, resulting in an increase in mechanical strength. In wet-extrusion, however, it does not appear to be the case; crystallinity is relatively constant for all draw ratios. This has also been observed by others.[16] During degradation of the

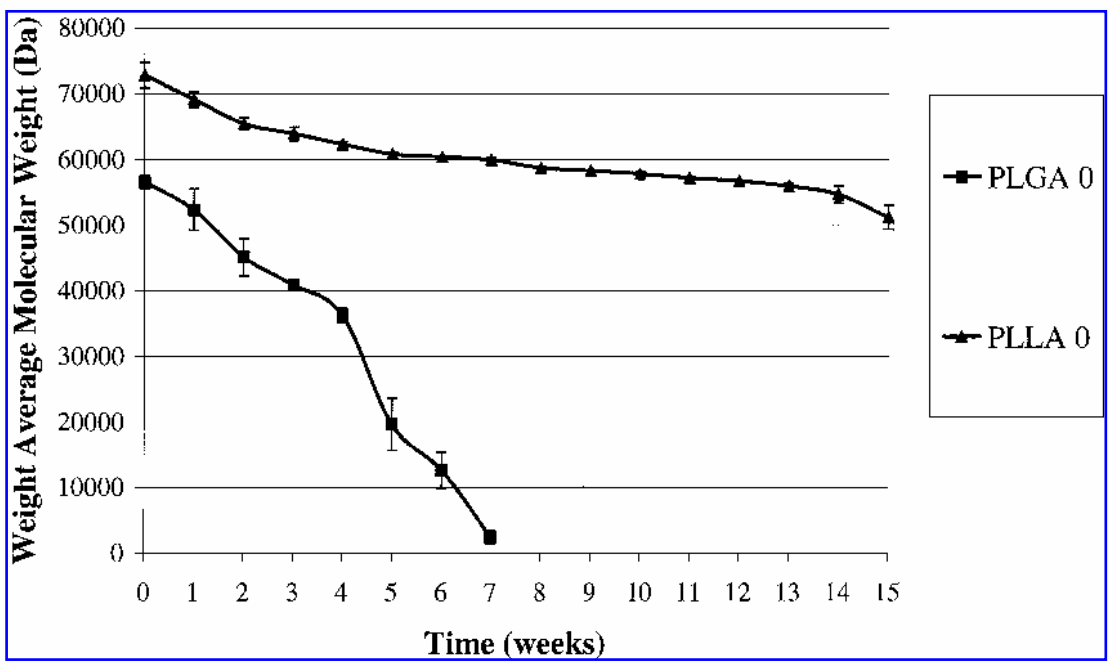

FIG. 4. Weight average molecular weight degradation is shown for fibers made from PLLA and from PLGA. These fibers were made by dissolving the polymer (PLLA at 10 wt/v%; and PLGA at 20 wt/v%) in methylene chloride and extruding it through a 22-gauge needle into hexane at a winding ratio of 42. Fibers were stored in 37°C PBS for up to 15 weeks. Molecular weight was determined by GPC against polystyrene standards.

20:1 draw ratio fibers, percent crystallinity decreased between weeks 4 and 6, but then increased between weeks 10 and 12. A parallel late increase in crystallinity was also seen with the 36:1 draw ratio fibers (Fig. 5).

In vitro *and* in vivo *biological results*

Ngo *et al*. and Nagarathnamma *et al*. have published papers using these fibers in the area of nerve regeneration.[13,14] These studies demonstrated that these fibers can be used both *in vitro* and *in vivo* with good biocompatibility. These fibers provided appropriate adhesion and contact guidance to axons as they extend down the fiber *in vitro*. Ngo *et al*. demonstrated axonal elongation across lesions in a rat sciatic nerve resection model over a distance of 18 mm, which is as long as has been reported in this model. This proved that these fibers possess good biocompatibility. There was only a mild inflammatory response as typically seen with PLLA.[1] In experiments with other cell types, including human dermal fibroblasts, rabbit corneal fibroblasts, human umbilical vein endothelial cells, and a retinal ganglion cell line (data not shown), all cell types adhered well, and as they proliferated they formed web structures spanning between adjacent fibers. This demonstrated that these wet-extruded fibers function as scaffoldings for numerous cell attachment and growth situations. We routinely use these fibers in the tissue-engineering laboratory course at our university.

DISCUSSION

Achieving fiber diameters of 30 μm or less was easy with appropriate choices of spinning conditions such as coagulation fluid and draw ratios (our unpublished data). The fibers were strong and had high elongation at break. We have varied choices of solvents, polymer concentrations, and draw ratios to find that a wide range of fiber properties is readily available.

The mechanical properties of these fibers were interesting. We speculated that the difference in properties seen between a 7.5 wt/v% fiber and an 8 wt/v% fiber was due to macrostructural changes in the polymer fiber. As the solvent diffused outward into the coagulation bath, and the coagulating bath fluid diffused into the polymer stream, the outer edge of the polymer stream became either polymer poor with dispersed aggregates of polymer-rich phases, or the outer edge became polymer rich with entrapped polymer-poor dispersed phases.

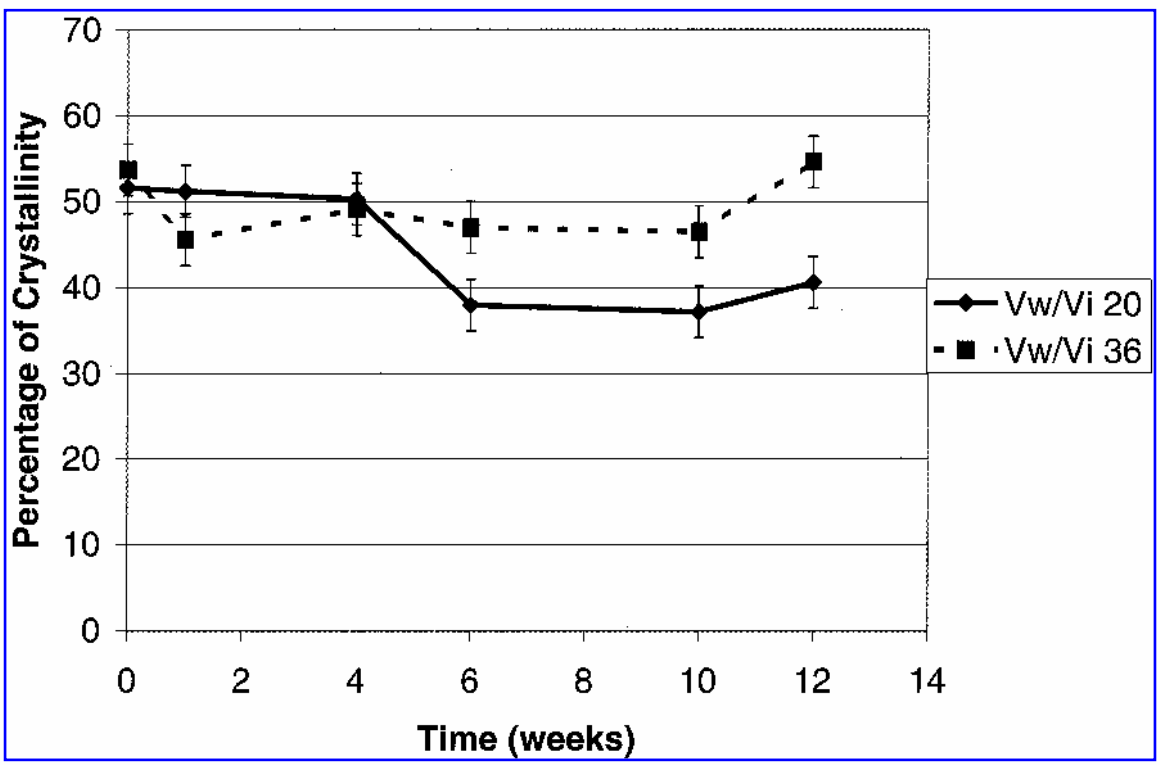

FIG. 5. Change in percent crystallinity as a function of time. PLLA fibers were made from a 10 wt/v% in chloroform solution and extruded into isopropyl alcohol with a 21-gauge needle. These fibers were incubated in PBS at 37°C for 12 weeks. Three samples were removed weekly and percent crystallinity was analyzed by DSC thermograms.

Case 1: polymer-poor continuous outer phase

If the outer edge became a polymer-poor continuous phase, there would be little barrier to diffusion of the coagulation bath fluid into the forming fiber. The dispersed polymer-rich phases would continue to contract and lose solvent. These polymer-rich dispersed phases would coalesce and form fibrils as the fiber fabrication proceeds. The result is noncircular cross-sectional fibers composed of compacted fibrils.

Case 2: polymer-rich continuous outer phase

In this case, the outside of the forming fiber would become polymer rich, forming a skin, which would provide increased resistance to diffusion of coagulation bath fluid, which is a nonsolvent, through this skin into or out of the polymer stream. The coagulation bath fluid that initially enters the polymer stream is entrapped and forms a polymer-lean dispersed phase, which would tend to coalesce during fiber formation into macrovoids trapped under the polymer-rich skin; however, the cross-section would remain substantially circular.

The data from our system with PLLA, chloroform, and isopropyl alcohol indicated that the initial polymer concentration of 7.5 wt/v% was described by case 1; whereas at 8 to 10 wt/v%, it appeared that case 2 is dominant. This is supported by SEMs of 7.5, 8, and 10 wt/v% fibers all extruded with an 18-gauge tip into isopropyl alcohol (Fig. 2C, B, and A, respectively). Note the coarse, noncircular appearance of the 7.5 wt/v% fiber (Fig. 2C), the intermediate texture of the 8 wt/v% fiber (Fig. 2B), and the relatively smooth, circular appearance of the 10 wt/v% fiber (Fig. 2A).

We also speculated that at draw ratios of 26:1 or higher (in our system), the polymer chains within the fiber would become essentially aligned. Therefore, in the absence of additional strain-induced crystallization (Table 1), there was little or no increase in mechanical strength with further increasing draw ratio.

ACKNOWLEDGMENTS

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