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Effects of protein molecular weight on the intrinsic material properties and release kinetics of wet spun polymeric microfiber delivery systems

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ABSTRACT

Wet spun microfibers have great potential for the design of multifunctional controlled release scaffolds. Understanding aspects of drug delivery and mechanical strength, specific to protein molecular weight, may aid in the optimization and development of wet spun fiber platforms. This study investigated the intrinsic material properties and release kinetics of poly(L-lactic acid) (PLLA) and poly(lactic-*co*-glycolic acid) (PLGA) wet spun microfibers encapsulating proteins with varying molecular weights. A cryogenic emulsion technique developed in our laboratory was used to encapsulate insulin (5.8 kDa), lysozyme (14.3 kDa) and bovine serum albumin (BSA, 66.0 kDa) within wet spun microfibers (~100 μm). Protein loading was found to significantly influence mechanical strength and drug release kinetics of PLGA and PLLA microfibers in a molecular-weight-dependent manner. BSA encapsulation resulted in the most significant decrease in strength and ductility for both PLGA and PLLA microfibers. Interestingly, BSA-loaded PLGA microfibers had a twofold increase (8 ± 2 MPa to 16 ± 1 MPa) in tensile strength and a fourfold increase (3 ± 1% to 12 ± 6%) in elongation until failure in comparison to PLLA microfibers. PLGA and PLLA microfibers exhibited prolonged protein release up to 63 days in vitro. Further analysis with the Korsmeyer–Peppas kinetic model determined that the mechanism of protein release was dependent on Fickian diffusion. These results emphasize the critical role protein molecular weight has on the properties of wet spun filaments, highlighting the importance of designing small molecular analogues to replace growth factors with large molecular weights.

1. Introduction

Micro- and nanoscale fibers have attracted considerable interest in the field of biomedical engineering [1–5]. In particular, microfibers prepared by wet spinning have been used as drug delivery devices and tissue engineering scaffolds due to their high surface-area-to-volume ratios for efficient drug release and their ability to be manipulated into a variety of complex macro-level scaffolds. To this end, the use of wet spun fibers as vehicles for drug delivery and biocompatible scaffolds for the assembly of regenerating tissue has been extensively studied. Controlled release of drug molecules has been achieved by encapsulating therapeutics within the polymer matrices of wet spun fibers [6–10]. Scaffolds made from a number of wet spun polymers, such as poly(lactic-*co*-glycolic acid) (PLGA), poly(L-lactic acid) (PLLA), polycaprolactone (PCL), and chitosan have also demonstrated the effectiveness of wet spun microfilaments as morphological guides for tissue regeneration [4,11–14].

Recently, controlled-release technologies and tissue engineering strategies have been combined for the regeneration of tissues, which require a complex sequence of biological cues and structural support [15–18]. Wet spun microfibers have great potential for the design of multifunctional polymeric systems. To date, the majority of research involving wet spun fibers focuses on the release of therapeutics or biocompatibility and tissue regeneration capabilities of three-dimensional scaffolds. Little is known about the effects of protein encapsulation on the material properties of wet spun fibers. Moreover, studies that have evaluated the mechanical properties of drug-eluting wet spun microfilaments focused only on small molecules (<1 kDa) such as levofloxacin and progesterone, or model proteins of similar molecular weight, such as BSA and ovalbumin [8–10,19]. Textile structures used for "next generation" tissue engineering strategies require structural support and controlled delivery of therapeutics with a wide range of molecular weights. In the past, our laboratory has shown that protein molecular weight influences drug release kinetics from PLGA microspheres [20]. To the best of our knowledge, this effect has yet to be evaluated in a wet spun fiber-based drug delivery platform.

The mechanical properties of drug-eluting microfibers are very important to their functionality as therapeutic scaffolds in the clin-

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ical arena. Mechanical properties of scaffold substrates have been shown to affect cell differentiation, proliferation and migration [21,22]. Mechanical strength can also alter host tissue integration; "mismatched" material properties can affect the maturation of regenerated tissues [18]. For clinical applications, the structural integrity of therapeutic implants is critical for surgical handling and implantation. Understanding the effect of protein encapsulation on the material properties of wet spun microfilaments may help predict the properties of macro-level scaffolds to optimize the function and growth of specific cell types. This paper contributes to the fundamental knowledge of the effects of protein molecular weight and encapsulation on the intrinsic material properties and release kinetics of wet spun microfilaments. Three proteins of varying molecular weight: insulin (5.8 kDa), lysozyme (14.3 kDa), and bovine serum albumin (66.0 kDa), were encapsulated into PLGA and PLLA microfibers by phase inversion, similar to previous studies from our laboratory involving microspheres [20]. Mechanical testing and thermal analysis were used to determine the effect of protein encapsulation and molecular weight on the material properties of wet spun microfibers. The influence of protein molecular weight on the release kinetics and mechanism of release was also characterized.

2. Materials and methods

2.1. Materials

Poly(L-lactic acid) (PLLA, i.v. = 1.04 dl g^{-1} in $CHCl_3$) (Lactel Biodegradable Polymers) and poly(D,L-lactic-*co*-glycolic acid) 75:25 ester terminated (PLGA,i.v. = 0.55–0.75 dl g^{-1} in $CHCl_3$) (Durect) were used in the fabrication of wet spun microfibers. Bovine serum albumin (Sigma), lysozyme (Sigma) and bovine zinc insulin (Gibco) were used as model proteins for this study. Dichloromethane (Fisher) and petroleum ether (Fisher) were the solvent and nonsolvent, respectively, used for wet spinning. Sorbitan trioleate (Span 85, HLB 1.8) (Sigma) was used in PLGA microfiber formulations. The micro BCA protein assay reagent kit used to detect protein concentration was used according to the manufacturer (Fisher).

2.2. Microfiber fabrication

Spin dope solutions were prepared using a modified cryogenic emulsion technique developed in our laboratory [23]. PLGA and PLLA microfibers were loaded with one of three proteins: bovine zinc insulin (INS, 5.8 kDa), lysozyme (LZ, 14.3 kDa), and bovine serum albumin (BSA, 66.0 kDa). Briefly, 0.5 ml of protein in ultra-pure water (20 mg ml^{-1}) was added to 10 ml of polymer in dichloromethane (DCM) (50 mg ml^{-1}), yielding an aqueous-to-organic phase ratio of 1:20. This two-phase system was vortexed for 60 s to create a meta-stable emulsion. The emulsion was immediately frozen in liquid nitrogen, creating frozen protein droplets dispersed in frozen dichloromethane/polymersolid solution. The frozen emulsion was lyophilized for 48 h at −100 °C. This process results in protein particles <2 μm embedded in a matrix of a polymer. Our experience with the water-in-oil (W/O) emulsion micronization method is that most proteins have a solid size <2 μm at the end of the process regardless of the nature of the protein (we refer here to the physical size of the particle, not the molecular weight of the protein) [24]. The dried polymer and protein product was then reconstituted in 2.5 ml DCM at a concentration of 200 mg ml^{-1} and placed into a gas-tight glass syringe fitted with a 22-gauge spinneret. A syringe pump was used to extrude the spin dope solution (0.02–0.06 ml min^{-1}) into petroleum ether at a solvent to nonsolvent ratio of 1:400, which resulted in the continuous formation of PLGA and PLLA monofilaments. PLGA formulations required 50 μl of Span 85 to prevent gelation around the spinneret during extrusion. Extruded microfibers were collected from the coagulation bath after all of the spin dope was extruded (~1.5 h residence time). Blank microfibers were also fabricated and used as controls. Duplicate batches of each formulation were made to ensure the reproducibility and uniformity of release profiles.

2.3. Scanning electron microscopy (SEM)

Lyophilized microfibers were mounted on adhesive metal stubs and sputter-coated with a 50–100 Å layer of gold–palladium (Emitech). Samples were viewed with a Hitachi S-2700 scanning electron microscope using an accelerating voltage of 8 kV. Micrographs were taken using a Quartz PCI digital imaging system. To determine the average cross-sectional area, microfibers were arranged into bundles onto paraffin film and rolled laterally into cylindrical tubes and orthogonally cut into thin discs. Five fields of view (15–22 filaments) of cross-sectioned microfibers were captured at 350× magnification and analyzed using ImageJ software (NIH).

2.4. Differential scanning calorimetry (DSC)

DSC measurements of blank and protein-loaded microfibers after fabrication (day 0) and incubation (day 63) were carried using a DSC-7 (Perkin Elmer) equipped with an Intracooler 2 intercooling system. Samples were subjected to: cooling to −25 °C, heating to 250 °C at 10 °C min^{-1}, cooling to −25 °C at 10 °C min^{-1}, and then reheating to the upper limit again at the initial rate. The crystallinity (X_c) of PLLA microfibers was calculated using

$$X_c = \frac{\Delta H_m}{93.7} \times 100. \quad (1)$$

where 93.7 J g^{-1} is the specific heat of melting of a 100% crystalline PLLA as reported in the literature [25].

2.5. X-ray diffraction (XRD)

The structural properties of PLLA formulations at time zero and 63 days after degradation were also determined using an automated X-ray diffractometer (Siemens Diffraktometer D5000) with a Cu K_α (λ = 1.54 Å) radiation. The diffraction angles (2θ) ranged from 6° to 60° with sampling intervals of 0.02° s^{-1}. Diffraction signal intensity was monitored and processed using DiffracPlus Software (Bruker AXS).

2.6. Mechanical testing

Uniaxial tensile tests were conducted using a materials testing system (Instron Model 4442) in accordance with the US Pharmacopeia absorbable suture testing standards [26]. Briefly, microfibers and yarns were secured to a paper frame (25 mm × 25 mm) and loaded into the crosshead clamps of the machine. Prior to loading, the sides of the paper template were cut leaving the sample intact. An elongation rate of 50 mm min^{-1} was applied until failure. The resulting load–displacement data collected by the digital acquisition system were converted to stress–strain data to calculate the ultimate tensile strength (UTS), percentage strain to failure and elastic modulus. The mechanical properties of microfibers were compared in SPSS v.19 (Chicago, IL) using a one-way analysis of variance (one-way ANOVA) test. Post hoc analyses for multiple comparisons were carried out using the Tukey multiple comparisons test. A P value of less than 0.05 was considered statistically significant.