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Multifunctional polymeric microfibers with prolonged drug delivery and structural support capabilities

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ABSTRACT

The strength and stability of hybrid fiber delivery systems, ones that perform a mechanical function and simultaneously deliver drug, are critical in the design of surgically implantable constructs. We report the fabrication of drug-eluting microfibers where drug loading and processing conditions alone increase microfiber strength and stability partially due to solvent-induced crystallization. Poly(L-lactic acid) microfibers of 64 ± 7 μm diameter were wet spun by phase inversion. Encapsulation of a model hydrophobic anti-inflammatory drug, dexamethasone, at high loading provided stability to microfibers which maintained linear cumulative release kinetics up to 8 weeks in vitro. In our wet spinning process, all microfibers had increased crystallinity (13–17%) in comparison to unprocessed polymer without any mechanical stretching. Moreover, microfibers with the highest drug loading retained 97% of initial tensile strength and were statistically stronger than all other microfiber formulations, including control fibers without drug. Results indicate that the encapsulation of small hydrophobic molecules (<400 Da) may increase the mechanical integrity of microfilaments whose crystallinity is also increased as a result of the process. Multifunctional drug-eluting microfibers can provide an exciting new opportunity to design novel biomaterials with mechanical stability and controlled release of a variety of therapeutics with micron-scale accuracy.

1. Introduction

Polymeric fibers have many medical uses, such as surgical sutures, dialysis devices, wound dressings and tissue engineering scaffolds. Advances in polymer and drug delivery sciences have led to the evolution of engineered fibers for use as drug delivery vehicles. Over the past decade, research towards the design of therapeutically active fibers has increased [1–6]. While there are many techniques to make polymeric fibers, only a small subset of these methods is suitable for drug encapsulation. Wet spinning is one such technique that lends itself to drug delivery technologies since it can be done at ambient temperatures and is most similar to conventional microsphere-based encapsulation techniques. Wet spinning also produces micron-sized fibers that have the potential to be woven, knitted, braided or embroidered into macro-level scaffold superstructures for the clinical reconstruction of damaged tissues/organs.

Wet spun microfiber delivery systems have been achieved by impregnating therapeutics into the core of hollow microfibers, entrapping therapeutics within microfibers, and chemically crosslinking or adsorbing therapeutics to the surfaces of microfibers. A broad range of biologically active therapeutics including antibiotics [7–9], heparin [10], proteins [6,11–14], growth factors [4,15], genes [16,17] and even viruses [18,19] have been incorporated into wet-spun microfibers. Recent efforts have focused on the biocompatibility and biological activity of therapeutics delivered from wet-spun microfibers. However, the effect of drug incorporation on the mechanical integrity of wet-spun microfibers is not well understood [9,20–22]. The mechanical properties of engineered microfibers for drug delivery and tissue engineering applications are important to their functionality in vivo. For clinical applications, therapeutic fibrous drug delivery implants must retain structural integrity during surgical implantation, and provide mechanical and pharmaceutical support in vivo throughout the process of tissue integration and implant degradation, which can occur over several months up to a year. Mechanical cues are also important for the organization, growth and maturation of reconstructed tissues. Even small changes in the mechanical properties of the local microenvironment have been shown to influence cell behaviors by altering cell–cell and cell–matrix interactions [23].

In this study, we examined the dynamic mechanical properties of drug-eluting wet-spun microfibers. Dexamethasone (DXM), a potent anti-inflammatory drug, served as the model therapeutic

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for this study. The local delivery of DXM has been shown to reduce adverse cellular immune responses to a variety of implanted biomaterials [24–28]. Targeted DXM delivery may be beneficial for reducing unwanted inflammatory responses to fibrous implants and improving tissue integration. Here we describe the drug–polymer interactions and the effects of DXM encapsulation and release on the physical and thermal properties of wet-spun poly(L-lactic acid) (PLLA) microfibers after up to 8 weeks of incubation.

2. Materials and methods

2.1. Materials

PLLA($M_w \sim 120{,}000$) was purchased from Lactel Biodegradable Polymers (Birmingham, AL). DXM and HPLC grade acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). Analytical grade petroleum ether (PE), dichloromethane (DCM), tetrahydrofluran (THF) and glacial acetic acid were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Fabrication of DXM-loaded microfibers

PLLA (7.5% w/v) DXM-loaded (1.0, 2.4 and 4.8 wt.%) and unloaded (0 wt.%) microfibers were wet spun by phase inversion. PLLA (526.9 ± 0.3 mg) was dissolved in a co-solvent ratio of DCM to THF of 6:1 (v/v). DXM was added to the co-solvent solution at varying concentrations up to 3.6 mg ml^{-1}, near its maximum solubility. The addition of THF was necessary to increase DXM solubility within the spin dope. The polymer/drug solution was loaded into a 5 ml syringe fitted with a 22-gauge spinneret and placed in a syringe pump with a solution flow rate of 0.06 ml min^{-1}. Since DCM and THF are miscible with petroleum ether, the immersion of the spinneret into the coagulation bath resulted in continuous microfiber formation and subsequent encapsulation of DXM. A rotating mandrel placed above the spin bath was used to collect microfibers for further experimentation. Each formulation in this study was characterized from multiple regions of meter-long microfiber bundles spun at the same time from one spin dope solution.

2.3. Microfiber morphology

Scanning electron microscopy (SEM) was used to study the surface and cross-sectional morphology of wet-spun microfibers. Fiber samples were placed on double-sided carbon tape and coated with a 50–100 Å layer of gold-palladium using a sputter coater (Emitech, Kent, UK). SEM was conducted using a Hitachi S-2700 (Tokyo, Japan) microscope with an accelerating voltage of 8 kV and a working distance of 12 mm. Micrographs were collected using a Quartz PCI digital imaging system. The average microfiber diameter was measured using NIH ImageJ software (Bethesda, MD) from 10 fields of view taken at ×100 magnification. The porosity was also measured from cross-sectioned microfibers and calculated as the pore area divided by the total cross-sectional area.

2.4. Determination of microfiber drug loading

Drug-loaded and control fiber bundles (5.0 ± 0.3 mg) were dissolved in 5 ml of 6:1 (v/v) DCM to THF until a clear solution was obtained. The encapsulation efficiency of DXM-loaded microfibers was determined by UV absorbance at 239 nm using quartz cuvettes to minimize background noise at the reading frequency. The percentage of drug encapsulated was calculated as the amount of DXM detected in microfibers relative to the total amount of drug added to the spin dope solution. Two samples from each formulation were assayed in duplicate.

2.5. DXM release study

As-spun microfibers from each batch (21.0 ± 2.0 mg) were incubated with 2 ml phosphate buffered saline (PBS, pH 7.4) in capped microcentrifuge tubes and kept at 37 °C. At each time point, 1 ml of the releasate was removed and replaced with fresh PBS. Prior to release analysis, DXM releasates were lyophilized and reconstituted in a mobile phase of 52:48 (v/v) 2 mM acetate buffer (pH 4.8) to acetonitrile. DXM detection was performed using a 3.9 × 150 mm Novapack C-18 column with a mobile phase flow rate of 1 ml min^{-1} at 240 nm after an average elution time of 2 min. Drug concentration was determined by comparing the area under the peak at the expected elution time with a calibration curve constructed from samples of known concentration.

2.6. Degradation study

PLLA microfibers (23.0 ± 2.0 mg) prepared by wet spinning were weighed in microcentrifuge tubes and incubated in 2 ml PBS (pH 7.4). The tubes were capped and placed at 37 °C. The duration of the degradation study was 8 weeks, with weekly terminal time points. At each sampling interval, the PBS solution was removed and the supernatant pH measured using a Corning pH meter (Medfield, MA). The remaining microfiber bundles were then washed three times in distilled water and lyophilized for 24–48 h.

2.7. Differential scanning calorimetry (DSC) and hyper-DSC analysis

The thermal properties of microfibers from terminal time points were analyzed for thermal transitions using a DSC-7 (Perkin Elmer) equipped with an Intracooler 2 intercooling system (Perkin Elmer). 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 reheating the sample to the upper limit again at the initial rate. Glass transition temperature, melting temperature and change in enthalpy of the melt were measured from the resulting thermograms. The per cent crystallinity (X_c) was also calculated using Eq. (1):

$$X_c = \frac{\Delta H_m}{\Delta H_{PLLA}} \times 100 \quad (1)$$

where ΔH_m is the enthalpy of melting of the samples and ΔH_{PLLA} (93.7 Jg^{-1}) is the specific heat of melting of a 100% crystalline PLLA as reported in the literature [29]. The dispersion of solid drug particles not solubilized within the polymer matrix was also analyzed using a DSC-8500 (Perkin Elmer) capable of hyper-DSC. Samples were subjected to heating from 20 to 310 °C at 200 °C min^{-1} and compared to the thermogram of free DXM from the manufacturer.

2.8. X-ray diffraction (XRD) analysis

The structural properties of PLLA microfiber formulations were 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).

2.9. Mechanical properties of PLLA microfibers

Uniaxial tensile testing was used to characterize the mechanical properties of wet-spun PLLA microfibers. Tests were performed at ambient temperature, humidity and pressure using an Instron materials testing machine (Model 4442). Gauge length and