

Incremental changes in anisotropy induce incremental changes in the material properties of electrospun scaffolds

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Abstract

Electrospinning can be used to selectively process a variety of natural and synthetic polymers into highly porous scaffolds composed of nano-to-m diameter fibers. This process shows great potential as a gateway to the development of physiologically relevant tissue engineering scaffolds. In this study, we examine how incremental changes in fiber alignment modulate the material properties of a model scaffold. We prepared electrospun scaffolds of gelatin composed of varying fiber diameters and degrees of anisotropy. The scaffolds were cut into a series of “dog-bone” shaped samples in the longitudinal, perpendicular and transverse orientations and the relative degree of fiber alignment, as measured by the fast Fourier transform (FFT) method, was determined for each sample. We measured peak stress, peak strain and the modulus of elasticity as a function of fiber diameter and scaffold anisotropy. Fiber alignment was the variable most closely associated with the regulation of peak stress, peak strain and modulus of elasticity. Incremental changes, as judged by the FFT method, in the proportion of fibers that were aligned along a specific axis induced incremental changes in peak stress in the model scaffolds. These results underscore the critical role that scaffold anisotropy plays in establishing the material properties of an electrospun tissue engineering scaffold and the native extracellular matrix.

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1. Introduction

The electrospinning process shows great potential as a gateway for the development and fabrication of physiologically relevant tissue engineering scaffolds [1–3]. A variety of native proteins [4–6], synthetic polymers [7–9], and blends of native and synthetic materials [10] can be selectively processed and electrospun into highly porous scaffolds composed of small diameter fibers. The physical, biochemical, biological and material properties of this unique class of materials can be regulated at several sites in the production process. Physical properties, including fiber diameter and

pore dimension, can be regulated by controlling the composition of the electrospinning solvent and the identity, concentration and/or degree of chain entanglements (viscosity) present in the starting polymer(s) [11,12]. The structural profile of the selected materials can even be modulated after the completion of the electrospinning process through nanofiber self-assembly events [13]. The biochemical profile of scaffolds can be manipulated through the addition of soluble growth factors [14] and other pharmaceuticals [15,16] during and/or after the electrospinning process. Biological properties can be further tailored to specific applications by controlling fiber identity and alignment.

Emerging evidence suggests that fiber alignment and overall scaffold anisotropy play a critical role in determining the material properties of electrospun materials

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[7,17,18]. However, efforts to examine how scaffold material properties develop as a function of these characteristics have been limited; developing an objective, quantitative measure of fiber alignment is a challenging task. In previous studies, we adapted and used the fast Fourier transform (FFT) to measure the relative degree of fiber alignment present in an electrospun scaffold [17,19]. We observed distinct material properties as scaffold anisotropy reached a critical threshold and here we extend our preliminary observations to define this threshold more precisely. In this study, we examine how fiber diameter and specific degrees of fiber alignment, as determined by the FFT method, interact with the testing angle to dictate the material properties of an electrospun scaffold.

To explore how fiber alignment regulates material properties, calfskin gelatin scaffolds of varying fiber diameters are electrospun onto a rectangular, grounded mandrel. Differing degrees of fiber alignment can be induced by rotating the mandrel at a constant rate between 0 and 6000 rpm [15]. Scaffolds are cut into “dog-bone” shaped samples (for materials testing purposes) in the longitudinal, perpendicular or transverse orientations. From these samples, the relative degree of fiber alignment, as measured by the FFT method, is determined. The samples are then tested to failure using a materials testing machine. From the data it appears that fiber alignment is the variable most closely associated with changes in scaffold material properties, and increasing the proportion of fibers aligned along a specific axis induces incremental changes in peak stress. These results emphasize the central role that scaffold anisotropy plays in determining the material properties of the native extracellular matrix and engineered scaffolds.

2. Materials and methods

2.1. Electrospinning

Reagents were purchased from Sigma–Aldrich (St Louis, MO) unless noted. Gelatin was suspended (120, 150 and 180 mg ml⁻¹) and agitated in 2,2,2 trifluoroethanol (TFE) for 24 h. Electrospinning suspensions were loaded into a 20 ml Becton Dickinson syringe capped with an 18-gauge blunt-tipped needle. The air gap distance between the source suspension and the grounded mandrel was set to 20 cm. A Harvard perfusion pump was used to meter the delivery of the electrospinning suspensions to the electric field. The positive output lead of a high-voltage supply (Spellman CZE1000R; Spellman High Voltage Electronics Corporation) was attached by an alligator clip to the blunt-tipped needle. For a detailed schematic of the electrospinning system, see Ref. [4]. Electrospinning was conducted at an accelerating voltage of 25 kV. The rate of solvent/polymer delivery was set at the maximal rate that did not induce dripping from the tip of the syringe. A stainless steel rectangular mandrel (75 × 20 × 6 mm) was used as a grounded target.

The target mandrel was regulated to rotate between 0 and 6000 rpm to induce varying degrees of alignment in

the electrospun scaffolds. A digital stroboscope (Shimpo Instruments DT3-11A) was used to continuously monitor the rotational speed of the target mandrel. Under the conditions of this study, fiber diameter remained constant over the range of mandrel rpm used to collect the scaffolds (not shown, see Ref. [17]). Samples were stored in a desiccation chamber to limit hydration prior to analysis.

2.2. Scanning electron microscopy

Average fiber diameter was determined from samples processed for conventional scanning electron microscopy (SEM, JEOL JSM-820). Dry, unfixed electrospun scaffolds were sputter coated with gold for imaging. SEM images were captured on Polaroid film, digitized via a flatbed scanner and analyzed with NIH ImageTool (UTHSCSA version 3). Average fiber diameter was determined from measurements taken perpendicular to the long axis of the fibers within representative microscopic fields (25 measurements per field). All measurements were calibrated from size bars incorporated into the SEM images at the time of capture ($N = 3$ from independent experiments).

2.3. Light microscopy

The scaffolds were imaged using a Nikon TE300 microscope equipped with a Nikon DXM 1200 digital camera. Bright-field images (3840 × 3072 pixels) were captured with a 20 × 0.40 NA bright-field objective lens. All images were archived as .TIF files.

2.4. Fast Fourier transform

As described in detail in a previous study, the FFT method was used to evaluate relative fiber alignment in electrospun scaffolds [17]. For a complete description of this method see Ref. [19]. The FFT function converts information present in an optical data image from a “real” domain into a mathematically defined “frequency” domain. The resulting FFT output image contains grayscale pixels that are distributed in a pattern that reflects the degree of fiber alignment present in the original data image. A graphical depiction of the FFT frequency distribution is generated by placing an oval projection on the FFT output image and conducting a radial summation of the pixel intensities for each angle between 0 and 360°, in 1° increments. The pixel intensities are summed along each radius and then plotted as a function of the angle of acquisition (position of the radial projection on the oval profile), usually between 0 and 180° (FFT data are symmetric so a pixel summation to 360° is unnecessary). The degree of alignment present in the original data image is reflected by the height and overall shape of the peak present in this plot. The position of the peak on the plot reports the principal axis of alignment.

For analysis, grayscale, 8-bit .TIF bright-field microscopic images were cropped to 2048 × 2048 pixels. FFT

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