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Influence of hydration on fiber geometry in electrospun scaffolds

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ABSTRACT

Finite element models of tissue engineering scaffolds are powerful tools to understand scaffold function, including how external mechanical signals deform the scaffold at the meso- and microscales. Fiber geometry is needed to inform finite element models of fiber-based tissue engineering scaffolds; however, the accuracy and utility of these models may be limited if they are informed by non-hydrated geometries. Scanning electron microscopy and confocal microscopy, coupled with Fourier analysis of the resulting images, were used to quantify how hydration alters fiber geometry in electrospun collagen and polycaprolactone (PCL) scaffolds. The results also quantify how image size affects fiber geometry. Hydration is demonstrated to increase fiber tortuosity, defined as the ratio of actual fiber length:end-to-end fiber length. For collagen scaffolds, hydration increased the mean tortuosity from 1.05 to 1.21, primarily from large ~2- to 10-fold) increases in smaller (<40 μm) wavelength amplitudes. For PCL fibers, the mean tortuosity increased from 1.01 to only 1.04, primarily from modest ~2-fold) increases in larger (>100 μm) wavelength amplitudes. The results demonstrate that mechanical simulations of electrospun scaffolds should be informed with hydrated scaffold geometries of at least 200 μm scale, in order to capture geometrical effects associated with fiber straightening.

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1. Introduction

The development of functional engineered tissues is a critical step in reducing the number of patients (around 18) who die in the U.S. every day while waiting for an organ transplant [1]. Unfortunately, the current generation of engineered tissues often fails to match the biological function and mechanical properties of native tissue. For example, engineered skin, although successful at closing large full-thickness burn wounds, does not recapitulate the full anatomy or strength of the native dermis [2] and remains several orders of magnitude weaker than normal full-thickness human skin [3,4]. As the body is a chemically and mechanically dynamic environment, it has been proposed that the static environment in which many tissues are cultured may not be sufficient to deliver the appropriate signals for more natural tissue development. This has motivated studies to better mimic in vivo conditions, with a particular focus on applying mechanical forces to the tissue during development [5–9].

In extracellular-matrix-rich engineered tissues, such as engineered dermis and tendon, mechanical signals are transferred from the external environment to cells via scaffold–cell interactions. To gain the most benefit from mechanical stimulations, it is critical to first understand and predict how a scaffold deforms under an applied load/extension and subsequently to understand how scaffold deformation controls cell deformation. For example, the type of scaffold material has been shown to play a large role in the cellular response of the same engineered tissue system. When mesenchymal stem cells were cultured in a collagen gel vs. collagen sponge and exposed to the same 2.4% strain profile for 12 days, only those within the collagen sponge produced a stronger, more organized engineered tendon [10]. Due to the virtually unlimited number of scaffold chemistry/architecture and mechanical stimulation profile combinations, the development of an in silico model of scaffold deformation would help to establish the role of initial scaffold geometry and material mechanics on the macro- and microscale scaffold response to external mechanical stimuli.

Finite element models of fiber-based tissue engineering scaffolds require fiber geometry and local fiber mechanical properties as input parameters. Several different geometric features and characterization methods have been used to furnish this information. Fiber diameter, angle, density and tortuosity are commonly quantified manually and with the aid of computer software [11–13]. Fiber orientation and anisotropy have been quantified using fully automated procedures such as mean intercept length, line fraction deviation, and the fast Fourier transform method [14–20]. This

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structural/geometric information has informed finite element (FE) models of poly(esteruretheaneurea) electrospun fibers, collagen gels and hydrogels [21–25]. However, the accuracy and utility of these models may be limited if they are informed by non-hydrated geometries. For example, hydration/sterilization can significantly change the macroscopic dimensions of electrospun scaffolds [26], leading to large inaccuracies in FE predictions [25,27].

This study quantifies the impact of using dry (as-spun) vs. wet (sterilized and hydrated) scaffolds on fiber geometries. Both natural (collagen) and synthetic (polycaprolactone) electrospun polymer fibers are considered and the consequences are evaluated for two different fiber lengths, $L = 40$ vs. 400 μm. Scanning electron microscope (SEM) images of dry fibers and confocal microscope (CM) images of wet fibers are used to furnish probability distributions for the Fourier amplitudes of fiber wavelengths ranging from $\lambda \sim 1$ to 400 μm. The implications are that geometric information from wet scaffolds over scales of at least 200 μm is needed to inform accurate mechanical models of fibrous scaffolds.

2. Materials and methods

2.1. Scaffold fabrication

Electrospun collagen scaffolds were fabricated using a 10% w/v solution of acid-soluble, type I collagen (SEMED S) and PCL scaffolds were fabricated using a 7.5% w/v solution of polycaprolactone (PCL, Sigma, St. Louis, MO; Mw 55,000–70,000 g mol^{-1}) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma, St. Louis, MO). Rhodamine (Sigma, Steinheim) was added to the scaffolds at a concentration of 0.1 wt.% to allow for fluorescent imaging. The collagen scaffolds were spun onto an 8.5 cm^2 grounding plate at a plate-to-needle distance of 20 cm and a potential of 30 kV. PCL scaffolds were spun onto the grounding plate at a plate-to-needle distance of 20 cm and a potential of 25 kV. Electrospinning conditions were set to produce fibers with a 0.5–1 μm radius. Collagen scaffolds were physically cross-linked immediately after electrospinning by vacuum dehydration at 140 °C for 24 h.

2.2. Scaffold area measurements

The electrospun scaffold area was measured before and after the sterilization and hydration process to assess macroscale changes in scaffold geometry. Collagen scaffolds were prepared for sterilization and hydration by chemical cross-linking in a 5 mM solution of 1-ethyl-3-3-dimethylaminopropylcarbodiimide hydrochloride (EDC, Sigma, St. Louis, MO) in 100% EtOH for 24 h [28]. Collagen and PCL scaffolds (36 mm biopsy punches, $n = 10$ per group) were then disinfected by soaking in 70% EtOH for 24 h and subsequently hydrated in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Research Organics, Cleveland, OH) buffered saline for 24 h. Using ImageJ, area calculations were performed on digital images of the scaffolds, taken in the as-spun condition and after processing and hydration. Average percent original area ± standard deviation after disinfection and hydration change was reported.

2.3. Scanning electron microscopy (SEM)

The morphologies of the electrospun collagen and PCL scaffolds were examined by SEM (QUANTA, FEI Co., Hillsboro, OR). Three 6 mm biopsy punches from the as-spun PCL scaffolds and the post-vacuum dehydration collagen scaffolds were removed from three different scaffolds, mounted onto aluminum SEM stubs with conductive carbon paint and sputter coated with gold–palladium. To ensure that no fiber melting or smoothing occurred, the current in the sputter coating system was kept below 18 mA and total coating time less than 60 s. Samples were imaged in secondary electron mode at 5 kV. Two magnifications furnished images with 20 μm vs. 200 μm fields of view, allowing the fiber morphology to be characterized over a range of length scales (a total of ten images per sample per length scale were collected). The fiber diameter from 25 fibers in each scaffold type (collagen-dry, PCL-dry) was measured using ImageJ.

2.4. Confocal microscopy (CM)

The morphology of dry and hydrated electrospun collagen and PCL fibers was examined using laser scanning CM (Olympus Flowview). For dry analysis, scaffolds were electrospun, cut into 1×1 cm squares, placed onto a glass microscope slide and overlaid with a coverslip. No mounting medium was used for dry imaging. For hydrated imaging, chemically cross-linked collagen and PCL scaffold punches were sterilized in 70% EtOH for 24 h, rinsed in PBS for 24 h, mounted onto glass microscope slides using Fluoromount G (Southern Biotech) and a coverslip placed over the sample. The rhodamine added to both the collagen and PCL electrospinning solutions caused them to fluoresce at an excitation wavelength of 543 nm, facilitating imaging with the laser scanning CM. Each scaffold was imaged to a depth of 10 μm using the Z-stack function with a 0.3 μm slice thickness. Images were taken with a 60× oil lens at two different magnifications, furnishing 20 μm vs. 200 μm fields of view. Scaffold images were processed in ImageJ, using filtration to select the most intense pixels. Also, the wet fiber diameter was calculated for 25 fibers from each scaffold (collagen-wet, PCL-wet) type using ImageJ.

2.5. Fourier spectral description of fiber geometry

A Fourier approach was used to describe single fiber geometries. This involved the selection of a fiber from an image and identifying two points A and B along it with separation distance $L = 200$ μm (Fig. 1, inset). A coordinate system (x, y) was defined with A at $(0, 0)$ and B at $(L, 0)$. The line AB along the x axis was divided into $N = 20$ equally spaced intervals of width Δx, with coordinates $x'_i = i\,\Delta x'$ ($i = 0$ to N). Points (x'_i, y'_i) were identified along the fiber (Fig. 1), with y'_i denoting the perpendicular distance from line AB to the fiber. The fiber was then described by the Fourier sine series:

$$y'(x') = \sum_{k=0}^{N-1} b_k \sin\left(\frac{k\pi x'}{L}\right) \tag{1}$$

where the Fourier sine coefficients were determined from the measured coordinates (x'_i, y'_i) according to [29]:

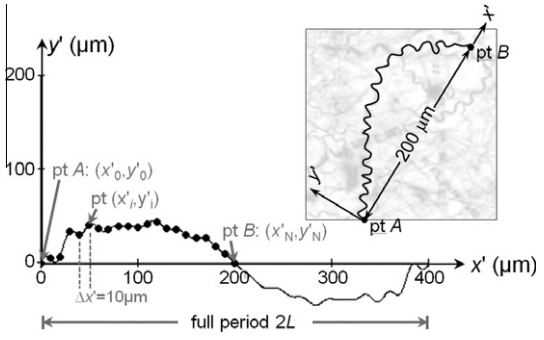

Fig. 1. An example wet collagen fiber geometry (fiber # 1.5) constructed from the digitized coordinates (dots) over the half period $L = 200$ μm; inset shows image and sample fiber with local x'–y' coordinate system.

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