Specimen Thickness Influences Material and Failure Properties of Electrospun Nanofiber Mats

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Disclosures:

Introduction: New fabrication techniques are being explored to biomimic and recapitulate the hierarchical structure of the natural collagen fibrous networks found in connective tissue such as tendon and ligament [1-3]. Electrospinning has gained considerable attention due to its ability to produce natural and synthetic fibers at the nanoscale with prescribed alignment and controlled geometries, which can be used to mimic the microenvironment of natural extracellular matrix (ECM) [4,5]. As is common practice, materials are frequently fabricated and characterized at one size, and based on the material properties gained from testing, are designed and fabricated into different geometries. This is perfectly acceptable, provided the material properties are conserved across a wide range of geometries. However, if that does not hold true, and the material properties vary with geometry, this assumption could lead to the design of constructs or tissue replacements with inferior mechanical performance and underpredicted resistance to failure, possibly resulting in premature construct/graft failure. This study aims to explore the influence of specimen thickness on the material and failure properties of electrospun nanofiber mats, both with aligned and randomly oriented fiber geometries. We hypothesize that, due in part to fiber kinematics, the properties will vary with thickness, and thus are not inherent to the material, but are coupled to specimen geometry. Such a finding can have significant impact on the design and characterization of fibrous biomaterials for orthopaedic applications.

Methods: Fabrication of Electrospun Nanofiber Mats: Aligned and randomly oriented electrospun nanofiber mats were made with a 12% (w/v) poly(e-caprolactone) (PCL, 80,000 MW, BrightChina Industrial Co., Ltd, Shenzhen, Guangdong, China) in a 3:1 chloroform to methanol (VWR International, LLC, Radnor, PA) solvent solution. This polymer solution was expelled from an 18 gauge needle attached to a glass syringe at 12 μL/min by a programmable syringe pump (BS-8000, Braintree Scientific Inc., Braintree, MA). Electrospinning was conducted with an applied 18 keV using a custom electrospinning apparatus. Nanofibers were collected on a spinning mandrel rotating at 500 rpm to achieve randomly oriented nanofibers or 2000 rpm to achieve aligned nanofibers. Electrospinning was conducted for 3, 5.5 and 10 hours to achieve the various thicknesses of samples. Resulting in aligned thin (18.64 μm), medium (52.18 μm), and thick (81.22 μm) as well as randomly aligned thin (17.88 μm), medium (36.18 μm), and thick (162.33 μm) thickness mats (±10 μm). Electrospun nanofiber mats were then removed from the mandrel and cut into individual samples using a 0.6 x 3.6 cm rectangular punch (C.S. Osborne & Co., Harrison, NJ). Aligned nanofiber samples were taken so that the fibers were aligned to the long axis of the sample.

Characterization: Fifty-four individual material samples (n=9 per group, 6 groups (3 thicknesses, aligned / random)) speckle-coated with an anisotropic, high-contrast pattern (for video strain measurements), then mounted in a Universal Materials Test Machine equipped with a 100 N load cell with 0.5 N resolution (Test Resources Inc., Shakopee, MN). Once secured in the pneumatic grips, each specimen was then given a slight positive preload (0.1 N), and stretched to failure at a constant rate (0.2 mm/s). All data were recorded at a rate of 50 Hz. A two-camera digital image correlation (DIC) system (Correlated Solutions, Inc., Columbia, SC) was employed to measure strains in the material, in a non-contacting manner. Video was collected at 10 frames per second, analyzed with VIC-3D 2010 DIC software (Correlated Solutions, Inc.), and a virtual extensometer was created for each specimen, in-line with long axis of the specimen. Cross-sectional thickness of samples was determined using a reflected light microscope (Micromaster Metallurgical Microscope, Fisher Scientific, Pittsburgh, PA) with optixCam summit software (The Microscope Store, LLC.).

Data Analysis: Raw, unfiltered data were analyzed. The peak load, ultimate tensile stress (UTS) and failure strain were found from the tensile load-elongation data. All samples exhibited a bimodal, linear response to elongation. Therefore, slopes of the stress-strain curve for each sample were used to determine both a short-range modulus (0.01 - 0.04 strain) and yield modulus (0.1 to 0.3 strain), with excellent agreement to the data (91.1±12.9). Data from the virtual extensometer, specifically the peak strain and linear strain rate were compared to the grip-to-grip displacement data. Statistical comparisons were assessed by general linear model ANOVA in Minitab 14 (α=0.05).

Results: Peak loads increased with thickness for both the aligned and randomly aligned samples, as expected for a structural property. However, when the influence of specimen geometry was removed, the peak stress decreased with thickness, with a more pronounced decrease exhibited in the aligned samples (Fig.1). Conversely, the failure strain was similar for all three thicknesses in each group (Fig. 1), and randomly aligned samples exhibiting a greater strain to failure than aligned mats. The short-range modulus and yield modulus displayed similar trends within each group. In the aligned group, both moduli were highest in the thin samples and lowest in the thick, with a significant decrease from thin to medium thickness, and only slight
decreases from medium to thick (Fig. 2). In the randomly aligned group, moduli were similar between thin and medium thicknesses, and significantly lower in thick specimens. Moduli were markedly (> 2-fold) higher in the aligned group than the random.

**Discussion:** In both aligned and randomly aligned mats, UTS, short-range modulus, and yield modulus were significantly influenced by specimen thickness, with highest values observed in thin specimens, and lowest values in the thickest. This highlights that the “material properties” (e.g., UTS, moduli) are not conserved over a range of thicknesses, and thus, are not truly an inherent property of the material, but rather are functions of specimen thickness. Perhaps even more striking, since scaffold materials are quite often characterized using specimens much smaller than their intended application (e.g., tissue construct or graft), and we observed UTS and moduli to decrease with specimen thickness, many scaffolds and/or tissue replacements may be designed using data that overpredicts failure resistance; possibly leading to inferior performance or premature rupture. These thickness-dependent properties could be due to friction and fiber-fiber interactions playing a significant role in thin specimens of only a few layers of fibers, and becoming less important as thickness increases, and the response is driven by the material’s bulk properties. However, the fact that this thickness-dependence is observed in the properties of both aligned and randomly aligned fiber geometries, indicates that these differences cannot be attributed solely to the reorientation of fibers during stretch.

**Significance:** These findings highlight that properties of fiber-based biomaterials may not be intrinsic to the material, but rather may depend on specimen geometry. This can have major impact on the characterization and design of fiber-based grafts and tissue engineered constructs.

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**References:**
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Fig. 1. Failure properties: (left) Ultimate tensile strength (UTS) decreases with increasing specimen thickness in both the random and aligned groups, however (right) failure strain is conserved within both groups across all three thicknesses. (# denotes greater than medium and thick; † denotes less than thin and medium; P<0.01).

Fig. 2. Yield Modulus decreases with increasing specimen thickness. Differences were significant across all thicknesses in the aligned group (denoted by *; P<0.01). In the random group, thick samples had significantly lower moduli than both thin and medium (denoted by †; P<0.01). Similar trends were observed in the short-range modulus values.