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Computational predictions of the tensile properties of electrospun fiber meshes: effect of fiber diameter and fiber orientation

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Abstract

The mechanical properties of biomaterial scaffolds are crucial for their efficacy in tissue engineering and regenerative medicine. At the microscopic scale, the scaffold must be sufficiently rigid to support cell adhesion, spreading, and normal extracellular matrix deposition. Concurrently, at the macroscopic scale the scaffold must have mechanical properties that closely match those of the target tissue. The achievement of both goals may be possible by careful control of the scaffold architecture. Recently, electrospinning has emerged as an attractive means to form fused fiber scaffolds for tissue engineering. The diameter and relative orientation of fibers affect cell behavior, but their impact on the tensile properties of the scaffolds has not been rigorously characterized. To examine the structure-property relationship, electrospun meshes were made from a polyurethane elastomer with different fiber diameters and orientations and mechanically tested to determine the dependence of the elastic modulus on the mesh architecture. Concurrently, a multiscale modeling strategy developed for type I collagen networks was employed to predict the mechanical behavior of the polyurethane meshes. Experimentally, the measured elastic modulus of the meshes varied from 0.56 to 3.0 MPa depending on fiber diameter and the degree of fiber alignment. Model predictions for tensile loading parallel to fiber orientation agreed well with experimental measurements for a wide range of conditions when a fitted fiber modulus of 18 MPa was used. Although the model predictions were less accurate in transverse loading of anisotropic samples, these results indicate that computational modeling can assist in design of electrospun artificial tissue scaffolds.

Keywords

fibrous mesh; network architecture; multiscale modeling; tissue microstructure

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1. Introduction

The goal of tissue engineering is to develop bioactive materials that, when implanted into tissue deficits, will integrate with the adjacent tissue, stimulate vascular infiltration, and initiate normal tissue remodeling. Current strategies to achieve such clinically effective materials have frequently involved incorporating biologic and pharmaceutical components into porous biomaterial scaffolds. Here, the selection of the biomaterial is based on biocompatibility, ease of processing into the desired three-dimensional architecture, and matching of the resultant mechanical properties with that of the target tissue (Butler et al., 2000). Polymers that have been widely tested include natural polymers, such as collagen, agarose, silk and fibrin (Enever et al., 2002; Grassl et al., 2002, 2003; Li et al., 2006; White and Deen, 2002), and synthetic polymers, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(ϵ -caprolactone) (PCL), and their co-polymers (Agrawal and Ray, 2001; Goldstein et al., 2001; Moran et al., 2003; Temenoff and Mikos, 2000), and polyurethanes (Hayashi et al., 1989; Matsuda et al., 2005). Concurrently, design of the scaffold architecture remains a challenging task. To be effective, the scaffold must be capable of regulating morphology and function of adherent cells, without compromising tissue-specific mechanical properties. In the case of anisotropic structural tissues (e.g. blood vessels, ligaments, and heart valve leaflets) achievement of suitable mechanical properties remains a critical challenge.

Recently, electrospinning has emerged as a promising method for fabricating fibrous scaffolds for tissue engineering applications such as blood vessel (Matsuda et al., 2005), anterior cruciate ligament (ACL) (Bashur et al., 2006), and cardiac tissue (Zong et al., 2005) (Kwon et al., 2005; Pham et al., 2006; Yang et al., 2005). Electrospinning involves the use of a high-voltage power supply to eject a polymer fiber from solution and deposit it onto a grounded target. Fiber diameter can be varied from 100 nm to more than 5 μ m by controlling the electrospinning conditions, and orientation of the resultant network can be imparted by depositing this fiber onto a moving target (Boland et al., 2006). Importantly, the micro-scale architecture affects both the morphology of adherent cells (Bashur et al., 2006), and the bulk mechanical properties of the scaffold (Boland et al., 2001; Boland et al., 2005). Therefore, the architecture must be engineered to guide cell behavior while remaining suitable for soft tissue engineering applications (Gupta et al., 2005; Nair et al., 2004).

The utility of electrospun fused fiber meshes, however, is limited by the absence of a theoretical framework with which to predict how the bulk mechanical properties depend on the properties of the individual fibers. Such a framework is inherently multiscale, with the scaffold dimension being on the *centimeter* length scale, while the underlying fibrillar architecture on the *micrometer* scale. The strong dependence of native tissue response on fiber orientation, diameter, and reorientation in response to strain (Billiar and Sacks, 2000b) suggests that consideration of both length scales is necessary, and thus, continuum constitutive models often cannot predict the tissue response under every loading condition (Humphrey, 2003). The scale separation makes multiscale, structure-based mathematical models an attractive option to describe the mechanical behavior of engineered tissues.

In this study, fused fiber meshes were electrospun from a degradable poly(esterurethane-urea) (PEUUR) elastomer, and fiber diameter and orientation were systematically varied. Mechanical testing was performed to determine experimentally the dependence of the elastic modulus on the mesh architecture, while a multiscale, structural model previously developed for modeling reconstituted type I collagen gels (Agoram and Barocas, 2001; Stylianopoulos and Barocas, 2007a) was employed to predict the elastic properties of the polyurethane meshes. Finally, the model was extended to predict the mechanical properties of an electrospun tubular mesh that might serve as a scaffold for an engineered blood vessel.

2. Methods

2.1 Materials

All chemicals including the 1,6-diisocyanatohexane (HDI), poly(ϵ -caprolactone) diol (average molecular weight 1250 Daltons, PCL1250), 1,3-propanediol bis(4-aminobenzoate (PDAB), diethyl ether, dibutyltin dilaurate (DBTDL), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), dimethyl formamide (DMF), and phosphate-buffered saline (PBS) were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. Anhydrous (< 50 ppm water) dimethyl sulfoxide (DMSO) was obtained from Acros Organics (Morris Plains, NJ). All chemical reagents were used as received except for PCL1250, which was dried for 24 hours at 80°C under vacuum (10 mm Hg).

2.2 Polyurethane synthesis

A segmented degradable PEUR elastomer was synthesized using standard techniques in a three-neck, round-bottom flask equipped with argon inlet and outlet, condenser, and stirrer (Guelcher et al., 2005). Anhydrous DMSO was charged to a round-bottom flask fitted with a condenser. HDI was added to the flask, which was then immersed in an oil bath at 75°C, purged with argon, and stirred with a Teflon blade stirrer turned by an electric motor. A solution of dried PCL1250 was charged into the reactor by means of an addition funnel. The NCO:OH equivalent ratio of the prepolymer was 2.0:1.0. The prepolymer content in the reactor was controlled at 14 wt%. DBTDL was added to the flask at 1000 ppm and the reaction was allowed to proceed for 3 h. A solution of chain extender in DMSO was prepared at 50°C and added to the resultant HDI.PCL1250.HDI prepolymer in the reaction vessel. The NCO:OH equivalent ratio of the polyurethane was controlled at 1.03:1.0 and the polymer concentration was 12 wt %. DBTDL was added to a concentration of 1000 ppm. The reaction was allowed to proceed at 80°C for 20 h. The polymer was then precipitated in diethyl ether and dried in a vacuum oven for 24 h at 80°C under 10 mm Hg vacuum. The density of the resultant polymer was determined to be 1.13 g/cm³ using a pycnometer (AccuPyc 1330, Micromeritics, Norcross, GA).

2.3 Electrospinning

PEUR was electrospun onto aluminum foil with controlled fiber diameters and degrees of orientation. To form isotropic meshes, aluminum foil was mounted on a stationary target and electrospun from a syringe equipped with a 22 gauge Teflon tipped needle using a 15 kV potential, a throw distance of 15 cm, and a syringe flow rate of 5 mL/h. PEUR concentrations of 11.0 and 13.5 wt% in HFIP were used to produce meshes with different fiber diameters. To form meshes comprised of partially oriented fibers, aluminum foil was mounted on a 6.2 cm diameter wire mesh drum and a 13.5 wt% PEUR solution was electrospun under the conditions described above. The drum was rotated at rates of 600 and 1300 rpm (corresponding to linear velocities of 2.0 and 4.4 m/s, respectively) to produce meshes with different degrees of fiber orientation. After electrospinning, the meshes were carefully peeled off of the aluminum foil for imaging and mechanical testing.

2.4 Scanning Electron Microscopy (SEM)

Fiber diameter and degree of orientation of fiber meshes were determined by analysis of SEM images. Briefly, electrospun meshes were mounted onto studs and sputtercoated with a 10 nm layer of palladium (Model 208HR, Cressington Scientific Instruments, Cranberry Township, PA). Images were acquired using a LEO 1550 Field Emission SEM (Carl Zeiss SMT, Thornwood, NY) operating at 5 kV with a 16 mm working distance. Resultant images were imported into ImagePro Plus software (ICube, Crofton, MD) for analysis of fiber diameter and orientation. The degree of alignment was characterized by the angular standard deviation as

described previously (Bashur et al., 2006). Low angular standard deviations denote more strongly aligned meshes. The diameter and orientation of at least 150 fibers were analyzed for each sample.

2.5 Mechanical Testing

The tensile modulus of the bulk PEUUR was determined from cast dogbones using ASTM D412-98a with modifications. Briefly, a 9 wt% solution of the PEUUR in DMF was prepared, and then heated in a 60 °C water bath overnight. The solution was cast in Teflon dish, and allowed to dry in a vacuum oven for two days. A punch was used to create dogbones with a 10 mm gauge distance and 2.7 mm gauge width. Thicknesses, measured using digital calipers, were 0.2 to 0.4 mm. The samples were soaked in PBS overnight and tested at 37 °C in PBS. The samples were pulled monotonically at a rate of 10 mm/min to 400% strain using a Tytron250 (MTS, Eden Prairie, MN). Modulus was measured from the linear range of the stress/strain curve. Results are presented as mean $\pm$ standard deviation for n=3 samples.

The tensile moduli of electrospun PEUUR meshes were determined using 15×10 mm strips that had been soaked in PBS overnight prior to testing. The thickness of the meshes was on the order of 0.1 mm and therefore could not be accurately measured with calipers. Instead thickness was estimated from measurements of mesh mass, length, width, and density (1.13 g/cm³) using an assumed porosity of 60% (based on published mercury intrusion porosimetry measurements (Kwon, 2005)). Cyclic testing was performed with a gauge distance of 10 mm, a frequency of 0.5 Hz, and a maximum strain of 18%. The temperature was maintained at 37 °C using a heated bath. Isotropic meshes were tested in one direction, and oriented meshes were tested both parallel and perpendicular to the direction of fiber alignment. A total of 220 cycles were performed, and the modulus was reported based on the last cycle. Modulus was calculated from the linear region of the stress strain curve (typically between 10 and 18% strain) using a cross-sectional area based on an assumed 60% mesh porosity. Results are presented as mean $\pm$ standard deviation for n=3 meshes.

2.6 Formulation of the Model

To accommodate the scale separation between the functional and structural scales of artificial tissues, a multiscale methodology was employed that incorporates the tissue microstructure directly. The scale-bridging method used in this study was the volume-averaging theory (Drew, 1971; Whitaker, 1986). A detailed description of the model can be found elsewhere (Chandran and Barocas, 2007; Stylianopoulos and Barocas, 2007a); a summary is given here.

The model is developed in two scales: the microscopic scale, which provides a statistical representation of the microstructure and is called representative volume element (RVE); and the macroscopic scale, which represents the whole tissue. The RVE consists of a three-dimensional fibrillar network that is intended to replicate the fiber diameter, density and orientation of an electrospun mesh (Fig. 1). In the network, wherever two fibers intersect, they are connected by a cross-link that allows free rotation of the fibers and the mechanical response of the fibers is governed by a constitutive equation. The macroscopic scale is modeled with the finite element (FE) method. Separate RVEs are constructed around the Gauss points of the FE solution, and the macroscopic deformation field determines the deformation of the RVE boundary through the FE basis functions. The deformation of the RVE compels the fibers to deform, and the solution of the force balance among the fibers determines the local forces developed on the RVE. From the local RVE forces and using volume-averaging theory, the averaged Cauchy stress tensor is calculated for use at the macro-scale (see Eq. 4). The averaged Cauchy stress tensor is calculated at each Gauss point of the FE domain and then the macroscopic stress balance is solved. The mathematical formulation is non-linear and requires iteration of the solution algorithm until convergence. The model accounts for the orientation

of the network, the interaction among the fibers, and the realignment of the fibers during deformation. These phenomena play a key role in the mechanical response of the material and vary considerably from tissue to tissue.

The RVEs were generated through a stochastic process (Stylianopoulos and Barocas, 2007b). Nucleation sites (seeds) were generated randomly inside a cubic space and gave birth to two segments that grew in opposite directions along a randomly chosen vector. The segments grew progressively by a unit length until they collided with the RVE boundary or with another segment. In the former case, a boundary cross-link was generated, and in the later case, collision between two segments was defined when their distance was less than a prescribed fiber diameter, and an interior cross-link was generated at the point of collision. In both cases the cross-linked segment stopped growing. A fiber was defined as the line between two cross-links associated with the same segment. Selecting the directional vectors from an anisotropic distribution allowed generation of networks with preferred alignments (Fig. 1b).

2.7 Governing Equations

The formulation described above requires a fiber constitutive equation for the microscopic scale, the equation that provides the averaged Cauchy stress, given by volume-averaging theory, and the stress balance for the macroscopic scale.

Most polymers are incompressible and neo-Hookean (Macosko, 1994), at least at moderate strains, and thus the polymer fiber constitutive equation used in this study is:

$$f = G_f A_f \left(\lambda^2 - \frac{1}{\lambda} \right) \quad (1)$$

where f is the force on the fiber, G_f is the fiber shear modulus, A_f is the cross-sectional area of the fiber, and λ is the fiber stretch ratio. For an incompressible material the elastic modulus, E_f , is three times as much as the shear modulus. To account for the decrease in the fiber cross-section in response to stretching, A_f was computed as $A_f = A_o / \lambda$, where A_o is the cross-sectional area of the unstretched fiber.

The volume-averaged Cauchy stress tensor, S , was obtained from the microscopic stress tensor, s (Nemat-Nasser and Hori, 1999):

$$S_{ij} \equiv \frac{1}{V} \int_V s_{ij} dV \quad (2)$$

where V is the RVE volume and index notation is used throughout. Taking into account that $s_{ij} = s_{kj} \delta_{ik} = s_{kj} x_{i,k}$, where δ_{ik} is the Kronecker delta and $\mathbf{x}$ is the directional vector, the divergence theorem implies that

$$S_{ij} = \frac{1}{V} \int_{\partial V} x_i t_j dS \quad (3)$$

where t is the traction exerted on the boundaries, ∂V , of the RVE. For a network of discrete fibers the integral of the traction is given by the forces on the boundary cross-links (Oda, 1999):

$$S_{ij} = \frac{1}{V} \sum_{\substack{\text{boundary} \\ \text{crosslinks}}} x_i f_j \quad (4)$$

A detailed derivation of the macroscopic stress balance is given in Chandran and Barocas (2007). We assume microscopic equilibrium, $s_{ij,i} = 0$, and use Leibnitz's theorem, to express divergence of the macroscopic stress tensor as:

$$S_{ij,i} = \frac{1}{V} \int_{\partial V} (s_{ij} - S_{ij}) u_{k,i} n_k dS \quad (5)$$

where u is the displacement of the RVE boundary and n is the normal unit vector. The right hand side of Eq. 5 is due to coupling between the non-uniform stress and the non-uniform deformation of the RVE boundary.

2.8 Comparison of the model to experimental measurements

In order to compare model predictions of the elastic modulus to those measured experimentally for PEUR meshes four model parameters had to be specified: fiber elastic modulus E_f , cross-sectional area A_o , the fiber volume fraction, θ , and the network orientation. Fiber cross-sectional area and the network orientation were calculated from experimental measurements of fiber diameter and the angular standard deviation and the fiber volume fraction was set to 0.4 – to match the assumed volume fraction of the electrospun meshes – and the elastic modulus, E_f , was determined by fitting the model to the experimental stress-strain response for meshes electrospun from an 11.0 wt% solution. RVEs were generated, and their angular standard deviations were checked to match the experimental values. The simulations were repeated for four networks for each case studied, and the average value of the elastic modulus was taken. Uniaxial extension simulations were performed for rectangular slabs up to 18% strain to permit comparison with experimental samples.

2.9 Extension to engineered blood vessels

The computational approach was employed to simulate inflation tests of an engineered blood vessel. The purpose was to demonstrate the utility of this computational framework for the design of a scaffold suitable for tissue engineering, and studies were undertaken to predict the effect of 1) fiber orientation and 2) mesh thickness on the mechanical response of the engineered vessel.

Polymeric scaffolds have been used to construct small-diameter vascular grafts (Matsuda et al., 2005; Vaz et al., 2005), and their mechanical properties have been compared to these of normal arteries (Hayashi et al., 1989; Sonoda et al., 2002). As a measure for the vessel stiffness, the stiffness parameter, β , proposed by Hayashi et al. (1980) has been used according to the following equation:

$$\ln(P/P_s) = \beta(D/D_s - 1) \quad (6)$$

where P is the pressure, D is the diameter of the vessel, and $P_s (=100 \text{ mmHg})$ and D_s are standard pressure and diameter, respectively. The stiffness parameter is calculated in the physiological pressures region (60–140 mmHg); a higher β value indicates a stiffer vessel.

Here, illustrative simulations of inflation tests were performed for vessel dimensions similar to those reported by (Matsuda et al., 2005). The simulated vessel had length 4 cm, thickness 250 μm , and inner diameter 3.0 mm. The model parameters were a fiber volume fraction, θ , of 0.4, a fiber diameter of 0.9 μm , and the elastic modulus, E_f , of 18 MPa. The vessel was inflated to 150 mmHg luminal pressure in 10 mmHg increments, and the expansion of the lumen was predicted. To study the effect of fiber orientation on the mechanical response of the vessel, three different fiber orientations were used: isotropic, axial, and circumferential. The isotropic network was circumferentially aligned with an angular standard deviation of 64° , while oriented networks were either axially and circumferentially aligned with an angular standard deviation of 40° . The model predictions were compared to the response of a typical human intracranial artery (Hayashi et al., 1980). The thickness of the simulated vessel was then varied to find the vessel thickness that exhibited the same mechanical behavior as the human artery. The stiffness parameter, β , was also calculated and compared to published data for native and engineered arteries.

3. Results

3.1 Comparison of the model to experimental measurements

Three cast dogbones of the PEUR were tested to 400% uniaxial strain and the elastic tensile modulus was determined from the slope of the linear part of the stress-strain curves. The average value of the elastic modulus was 2.7 MPa.

The experimental results are summarized in Table 1 and Table 2. Table 1 shows the elastic modulus for isotropic meshes prepared from concentrations of 11.0 and 13.5 wt% in HFIP. The data suggest that the elastic modulus of the scaffolds is sensitive to the polymer solution concentration. Table 2 shows the experimental measurements for 13.5 wt% concentration with 0, 2.0, and 4.4 m/s drum velocities. The moduli parallel ($\parallel$) and perpendicular ($\perp$) to fiber alignment are also presented. Here, the modulus parallel to the fiber alignment increased systematically with decreasing angular standard deviation while the modulus perpendicular to the fiber alignment decreased with increasing alignment.

The computational model was fit to the stress-strain curve for a PEUR mesh electrospun from an 11.0 wt% solution (Figure 2). The resultant best fit for the fiber Young's modulus, E_f , was found to be 18 MPa (Fig. 2), which is more than six times greater than the measured modulus for the solution-cast polymer. The computed moduli are presented in Fig. 3 and Fig. 4. At the same figures the experimental moduli, as given in Table 1 and Table 2, are plotted in order to illustrate the comparison between theory and experiment. The predicted moduli for isotropic meshes were 0.64 MPa for a fiber diameter of 0.53 μm and 0.90 MPa for a fiber diameter of 0.9 μm . The predicted moduli for 41.2° angular standard deviation (2.0 m/s drum velocity) were 1.14 MPa ($\parallel$) and 0.37 MPa ($\perp$) and for 29.9° angular standard deviation (4.4 m/s drum velocity) were 2.60 MPa ($\parallel$) and 0.33 MPa ($\perp$). Here, the computed moduli of the meshes is lower than that of an individual fiber, E_f , because of volume fraction and network effects. From the comparison of the predicted and experimental moduli we conclude that the model predictions of the modulus were not significantly different from the experimental data for the isotropic meshes and the modulus parallel to the fiber direction for the oriented meshes ($p > 0.77$ in all cases). However, the model significantly underpredicted the tensile moduli perpendicular to the direction of stretching for the oriented meshes ($p < 0.01$).

3.2 Extension to engineered blood vessels

The predicted pressure-diameter response of the vessel for the three different orientations is shown in Fig. 5. In the same figure, the pressure-diameter response of a typical human intracranial artery is also shown (Hayashi et al., 1980). The shadowed area in the plot indicates

the physiological pressure range. The model predicts that an axially oriented vessel is the most compliant, and the vessel stiffens with increasing circumferential orientation. The model predicts that PEUR vessels would not follow the low-pressure response of normal arteries, which are very compliant because of the uncrimping and reorganization of the collagen fibers (Roach and Burton, 1957). At physiological pressures, however, the collagen fibers are under tension and the artery is stiffer than that predicted for a PEUR vessel.

Next, the wall thickness of the circumferentially oriented vessel was increased to 1000 μm to match the stiffness of the human intracranial artery (Fig 6). The stiffness parameter (β), as calculated from the predicted pressure diameter curves of Fig. 6 using Eq. 6, was 15.8 for 1000 μm wall thickness, as compared to 9.1 predicted for 250 μm thickness PEUR vessel and 21.2 for a normal intracranial artery. For comparison, the thickness of the human intracranial artery is 100 μm (Hayashi et al., 1980), which is thinner than either PEUR vessel models.

4. Discussion

The tensile properties of electrospun polyurethanes were measured as a function of fiber diameter and orientation, and compared to those predicted using a mathematical model for fibrous networks. The experimental results revealed that the tensile properties of fibrous meshes are sensitive to the microstructural architecture. Aligned samples showed mechanical anisotropy consistent with their structural anisotropy. In particular, the moderately aligned samples (2.0 m/s drum velocity) exhibited a ratio of 2.5 between parallel and perpendicular modulus, while the more highly aligned samples (4.4 m/s drum velocity) exhibited a ratio of 5.4. Computational model predictions of the tensile properties were in good agreement with the experimental measurements for isotropic meshes and for anisotropic meshes in the direction parallel to fiber orientation. However, the model under-predicted the experimentally determined modulus for anisotropic meshes in the direction perpendicular to fiber orientation.

Simulated inflation tests of tubular meshes were performed to illustrate the utility of our computational approach to the design of engineered blood vessels. Here, our objective was to apply the model to predict the structure of the PEUR scaffold (vessel thickness and network orientation) that would match the strength of normal tissue. The studies showed that a circumferentially-oriented network is stiffer than the other two network orientations tested, as was expected. In addition, the model predicted that a circumferentially-oriented PEUR network would need to be 1000 μm thick to match the pressure-diameter response of a typical human intracranial artery. However, the model predicts that such a mesh would not possess the toe in mechanical response intrinsic to blood vessels.

The stiffness parameter β of the simulated PEUR vessels ranged from 9.1 to 15.8 depending on the wall thickness. Table 3 presents the β -values predicted by the model along with experimental measurements for vessels made from polyurethane and for native arteries. The model predictions are within the range of β -values reported in experimental studies for polyurethanes. Furthermore, comparison between the stiffness of native and simulated arteries suggests that polyurethane grafts can be designed with the mechanical properties of soft arteries. To achieve the properties of relatively stiff arteries, such as the intracranial artery considered in this study, the model suggests that the mesh thickness be increased and fiber orientation altered. However, additional options that could be tested readily using this model include increasing the fiber modulus – to simulate stiffer biomaterials (e.g., PLCL (Inoguchi et al., 2006) or PTFE (Sonoda et al., 2002)) – and decreasing mesh porosity.

The agreement of model predictions with experimentally determined mesh properties provide insights into the mechanics of electrospun networks under uniaxial strain, while discrepancies reveal limitations of the model framework. In particular, two discrepancies were identified.

First, although a PEUUR modulus 2.7 MPa was measured for cast films, a fiber modulus, E_f , of 18 MPa was required in order to match the model prediction to the experimental data in Fig. 2. Second, although the model was able to accurately predict moduli parallel to fiber alignment, it underestimated moduli perpendicular to fiber alignment. Both of these discrepancies may have resulted from assumptions that were built into the computational model as well as differences between the RVEs and real electrospun meshes structures.

Two intrinsic assumptions of this model – which was originally developed for collagen fibers (typically 50–200 nm in diameter) – are that bending forces are negligible and that fibers can rotate freely at cross-links. These assumptions result in a model that considers only tensile strain, and therefore should generate predictions of mesh modulus that are independent of fiber diameter. Interestingly, the computational model predicts an increase in modulus with increasing fiber diameter (Figure 3), but this increase likely reflects a decrease in the angular standard deviation (Table 1). Because beam mechanics predicts that bending force scales as fiber thickness cubed, incorporation of fiber bending into our model should cause the mesh modulus to increase with increasing fiber diameter. Evidence of this trend has been observed previously (Boland et al., 2001; Boland et al., 2005) although contrary data has also been reported (Huang et al., 2004). A more detailed analysis of the mechanics of fiber networks, where fiber bending was incorporated, can be found in Wang et al., 2000 and Wang and Sastry, 2000. In those studies, Euler-Bernoulli beam theory was used for fiber length/diameter aspect ratios greater than 5, while for lower aspect ratios Timoshenko beam theory was employed to account for deformation due to transverse shear. According to Head et al., 2003 bending dominates in networks that possess a low fiber density or that consist of relatively short fibers. In contrast, networks with high fiber density or long fiber length are dominated by fiber stretching. Incorporation of bending into our model is expected to increase the stiffness of the scaffolds, and would likely result in a lower value for the fitted parameter E_f . In addition, because fiber bending is more pronounced when the meshes are stretched perpendicular to their direction of alignment, incorporation of bending into our model might increase predictions of modulus perpendicular to fiber alignment.

The properties of fiber cross-links (e.g., geometry, restriction to fiber motion) also can affect the modulus of the mesh. Berhan and Sastry, 2003 showed that the geometry of a cross-link and the angle that is formed between two connected fibers affect the network forces under loading. In addition, fixed cross-links provide additional rigidity to fiber networks when fiber bending is not negligible (Wilhelm and Frey, 2003, Head et al., 2003). In our model we permitted free rotation at cross-links. This likely diminished the predicted modulus of our meshes, especially in the case of strain applied perpendicularly to fiber orientation, where reorientation of the fibers is expected to be significant.

Finally, the model predictions can be affected by the equation used to describe the response of the fiber (Eq. 1). In this study we treated the PEUUR fibers as a neo-Hookean material based on our experience on modeling polymer networks. Alternatively, phenomenological exponential expressions have been used (e.g., Stylianopoulos and Barocas, 2007a, 2007b) that predict an almost zero force in compression to account for buckling of the fibers. The incorporation of fiber buckling into our model is not expected to affect significantly the tensile mechanical properties of the scaffolds, particularly for the properties parallel to the direction of strain as buckling is expected to occur primarily in the transverse direction.

In addition to limitations with the model assumptions, differences between model and experimental data may also have stemmed from our inability to determine accurately the properties of our electrospun mesh, including cross-link density, spatial homogeneity of mesh architecture, and mesh porosity. From SEM images (Fig. 1) the diameter and orientation of individual fibers can be readily determined and used to generate RVEs. However, cross-links

between adjoining fibers cannot be unambiguously identified, and consequently their densities could not be incorporated into the design of RVEs. In addition, the RVEs were generated based on SEM images of the topmost fibers in a mesh. Microarchitecture (e.g., fiber diameter, orientation, angular standard deviation) was assumed to be uniform throughout the mesh thickness, but spatial homogeneity of mesh architecture was not verified. Finally, the fiber volume fractions of the PEUUR meshes were not determined. Instead, volume fraction was assumed to be 40% for all samples, based on the mercury intrusion porosimetry data of Kwon, 2005.

Another limitation of this study is the small n value in the test data ($n=3$). A larger sample size might have resulted in more accurate measurements for the mechanical properties of the meshes and would allow us to determine better the model accuracy.

The inclusion of a fitted parameter, E_f , in the model allowed us to compensate for limitations in the model assumptions as well as differences between the RVEs and real fiber meshes. In particular the high fitted value, $E_f=18$ MPa, relative to the PEUUR modulus, $E = 2.7$ MPa, suggests that fiber bending and rigidity at fiber cross-links may not be negligible. In addition, this discrepancy suggests that the volume fraction and cross-link density of the RVEs are underestimates of the true mesh properties. We must note, though, that the differences between the bulk modulus and E_f may have stemmed from differences in the processing conditions used to produce solution cast films and electrospun meshes. Mechanical processing techniques, such as fiber spinning and film drawing are known to produce anisotropy in mechanical properties (Wilkes, 2003), and electrospinning – in particular – has been shown to cause strain-induced hardening (Gu et al., 2005).

To conclude, the mechanisms that govern the macromechanical behavior of tissues and tissue constructs is not yet well understood. In our work, we permitted non-affine fiber deformations, based on research on network mechanics (Chandran and Barocas, 2006), and the position of the fibers was determined by the solution of a force balance. Here, the accuracy of the model predictions depends largely on the micromechanics of the fiber network (e.g., fiber stretching, bending, buckling, cross-link bending). This approach differs from affine deformation models (e.g., Billiar and Sacks, 2000a; Driessen et al., 2005; Zulliger et al., 2004) in which fibers do not interact with each other but are treated as a collection of independent units embedded in the tissue matrix. In affine models, the fibers rotate and stretch homogeneously with the macroscopic deformation and the model predictions depend only on the fiber mechanical properties. Although non-affine models are computationally more intensive, they should be more accurate because they account for microscopic network effects. Their accuracy, nevertheless, depends on the extent to which they capture the micromechanics of the network.

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Figure 1. SEM images and model representations of electrospun meshes. a) Unoriented mesh formed from an 11 wt% solution onto a stationary target. Angular standard deviation is 67.5° and scale bar is $1\mu\text{m}$. b) Oriented mesh formed from a 13.5 wt% solution onto a target rotating at 2.0 m/s . Angular standard deviation is 41.2° and scale bar is $10\mu\text{m}$.

Figure 2. Experimental (squares) and computed (curve) stress-strain curve for PEUUR mesh electrospun from 11 wt% solution in HFIP.

Figure 3.

Experimental (black bar) and computed (white bar) moduli for PEUUR meshes electrospun from 11 (diameter 0.53 μm) and 13.5 wt% (diameter 0.90 μm) solutions at stationary drum. The standard deviations are also depicted.

Figure 4.

Experimental (black bar) and computed (white bar) moduli for PEUUR meshes electrospun from 13.5 wt% solution at rotation rates of 2.0 m/s (41.2°) and 4.4 m/s (29.9°) parallel (Fig. 4a) and perpendicular (Fig. 4b) to fiber alignment. The standard deviations are also depicted.

Figure 5. Initial and inflated at 150 *mmHg* luminal pressure configuration of the blood vessel (left). Pressure vs. inner diameter response of the vessel for random (squares), axially oriented (circles), and circumferentially oriented (triangles) meshes, and for a human intracranial artery (Hayashi et al., 1980) (solid).

Figure 6.

Pressure vs. inner diameter response for circumferentially oriented meshes for 250 μm (triangles) and 1000 μm (crosses) wall thickness, and for an intracranial artery (Hayashi et al., 1980) (solid).

Table 1

Measured fiber diameter, angular standard deviation and elastic modulus for PEUUR meshes electrospun from 11 and 13.5 wt% solutions onto a stationary target. Mean and standard deviations for fiber diameter and angle were calculated from $n \geq 150$ individual fibers. Mean and standard deviation for modulus were calculated for the 220th cycle for $n=3$ meshes.

Concentration in HFIP (wt%)	Fiber diameter (μm)	Angular standard deviation ($^{\circ}$)	Modulus (MPa)
11.0	0.53 ± 0.26	67.5	0.63 ± 0.27
13.5	0.90 ± 0.49	54.6	0.89 ± 0.44

Table 2

Measured fiber diameter, angular standard deviation and elastic modulus for PEUUR meshes electrospun from 13.5 wt% solution at different rotation rates. Modulus was determined both parallel (||) and perpendicular (⊥) to the direction of rotation. Mean and standard deviations for fiber diameter and angle were calculated from $n \geq 150$ individual fibers. Mean and standard deviation for modulus were calculated for the 220th cycle for $n=3$ meshes.

Speed (m/s)	Fiber diameter (μm)	Angular standard deviation ($^\circ$)	Modulus (MPa)
Stationary	0.90 ± 0.49	54.6	0.89 ± 0.44
2.0	0.63 ± 0.30	41.2	1.41 ± 0.81
2.0 ⊥	0.63 ± 0.30	41.2	0.57 ± 0.17
4.4	0.57 ± 0.29	29.9	3.04 ± 1.87
4.4 ⊥	0.57 ± 0.29	29.9	0.56 ± 0.16

Table 3
 β -values predicted by the model and measured for different materials.

vessel type	polyurethane (model)	polyurethane (Hayashi et al., 1989; Matsuda et al., 2005)	carotid (Hayashi et al., 1989)	femoral (Hayashi et al., 1989)	intracranial (Hayashi, et al., 1980)
β	9.1–15.8	8.4–29.5	5.25	19.84	21.2