INTRODUCTION
Synthetic scaffolds for heart valve tissue engineering applications require mechanical properties comparable to the native valve leaflet tissue for at least the minimum time necessary for the seeded cells to lay down an equivalent supporting matrix (Fig. 1). To achieve maximal results in the scaffold design process, it is beneficial to know and control specific scaffold characteristics that may alter the mechanical properties of the scaffolds so that a minimal amount of parameters could be changed to achieve the characteristics of the desired scaffold (i.e. for isotropic, moderately anisotropic, or highly anisotropic tissues). One familiar characteristic in electrospun scaffolds is fiber tortuosity, which is analogous to collagen crimp in valvular tissues. One can delay the onset of scaffold stiffness by controlling the degree of tortuosity and the fiber angles at which the tortuosity is more (or less) prominent. A constitutive model that incorporates the effects of these scaffold characteristics, and that can predict the response of the scaffold without having to perform time-consuming mechanical tests, would assist in the design of the scaffold and allow for more expediently-designed and predictable custom-tailored scaffolds. In this study, we extended our previous research with electrospun poly ester (ureth ane) urea (ePEUU)s scaffolds to incorporate the effects of fiber tortuosity on the mechanical response of the scaffolds. We also developed a constitutive model that is dependent not only on fiber angle but also on the fiber tortuosity with respect to fiber angle.

MATERIALS AND METHODS
Electrospun Scaffolds. The methods used to develop the ePEUU scaffolds have previously been reported [1]. In brief, three generators were employed with 12 kV charging the steel capillary containing the polymer solution, –7 kV charging the aluminum collection mandrel, and 3 kV charging a steel mesh screen, which acted to control the area of fiber deposition onto the aluminum mandrel. The PEUU was synthesized at a 5% wt concentration in hexafluoroisopropanol under mechanical stirring at 25°C. The PEUU solution was fed by syringe pump into the steel capillary (I.D. = 0.047) suspended vertically over the center of the cylindrical steel mesh focusing screen and aluminum rotating mandrel (4.5” diam.) The mandrel speed was varied between 0.3 and 14.0 m/s. Mechanical Testing. Biaxial testing was performed on the ePEUU scaffolds. The procedures for biaxial testing have been previously reported [1]. The specimens were tested in room-temperature water at equibiaxial tensions (T11:T22) up to 90 N/m on each side, with ten cycles. The testing protocol consisted of T11:T22 test ratios of 0.1:1.0, 0.5:1.0, 0.75:1.0, 1.0:1.0, 1.0:0.75, 1.0:0.5, and 1.0:0.1.

Figure 1. (a) Biaxial mechanical specimen location of a pulmonary heart valve leaflet. (b) Resulting biaxial mechanical properties along with those of a 2300 RPM ES-PEUU scaffold.

Structural Characterization. To determine the degree of alignment for the scaffolds, SEM images were obtained for the various spin speeds and analyzed using a custom image analysis software routine written in MATLAB. Two masks are created and convolved with every pixel in the image, leading to a gradient vector and angle associated with each pixel [1]. A boxsize (dimensions depending on the width of the fiber diameters) is chosen and the weighted contribution from each pixel is calculated using an accumulator function and assigned to that region. Several images were taken for each spin speed and then analyzed, with the resulting orientation data then being combined to determine the overall fiber orientation for the specimen. Tortuosity Measures. Tortuosity measures were performed on the SEM images of all unstrained scaffolds by tracking a fiber for the viewable length of the fiber (Fig. 2). Tortuosity was calculated by dividing the full length of the fiber by the end-to-end distance of the fiber. Twenty-five fibers were tracked on each SEM image for a total of 150 fibers for each spin speed. To better understand the change in tortuosity with deformation, a stage was designed that allowed the scaffold to be stretched and imaged using SEM. Specific regions of the scaffolds were imaged in a...
unstretched reference and deformed state.

Figure 2. Tracking of fibers to determine the degree of tortuosity with respect to angle

The resulting fiber orientation data were normalized using,

\[ R(\theta) = \frac{T(\theta)}{\sum_{\theta}^{\pi/2} T(\theta) \Delta \theta} \tag{1} \]

where \( \theta \) is the fiber direction with respect to the preferred fiber axis. The PEUU effective fiber stress-strain properties were determined from the mechanical data using,

\[ S_f(\theta) = K \int_0^\varepsilon D(x,\theta) \frac{\varepsilon - x}{(1 + 2x)^2} \, dx \tag{2} \]

where \( S_f \) is the 2nd Piola–Kirchhoff fiber stress, \( K \) is the fiber elastic modulus and \( D(x,\theta) \) is the statistical distribution accounting for the fiber recruitment. \( D(x,\theta) \) is a function of the fiber strain and orientation \( \theta \), with the strain itself a function of fiber angle, since it was found that tortuosity varies with angle as the mandrel speed increases (Fig 2). The structural and mechanical data were then combined to determine the Lagrangian membrane stresses. Using the structural data, \( R(\theta) \), and a single equibiaxial test for the determination of the fiber stress-strain response, the model allows one to predict the complete biaxial mechanical response of the polymer.

RESULTS

The PEUU scaffolds displayed higher orientation with increasing stretch as tortuosity is gradually lessened. Tortuosity was higher in the direction of spin of the mandrel. The scaffolds with the most amount of variation in tortuosity were those developed at higher mandrel velocities (fig 3). Tortuosity for random (isotropic) scaffolds exhibited no dependence on fiber angle. As the mandrel speed was increased though, tortuosity was much more dependent on fiber angle. Inclusion of the tortuosity data into the constitutive model yielded a much more robust fit to the experimental data. Upon stretching the scaffolds and viewing them under the SEM, it was seen that the fibers gradually straightened (fig 4). The disappearance of tortuosity depended on the scaffold, which showed angular dependence, and also the amount of stretch. This is the first time that this angular dependence has been incorporated into a structural model developed for soft tissues or scaffolds. Also shown are dual photon images of the ES-PEUU scaffold demonstrating that this imaging technique can be utilized to quantify scaffold fiber rotation and stretch, as well as cellular deformations (Fig. 5).

CONCLUSIONS

The biaxial stretching analysis allowed for the measure of tortuosity fibers and the gradual diminishing of that tortuosity with stretch. The data allowed for a more robust constitutive model to predict the mechanical response of the scaffolds. Future work will investigate the effect of stretch on cells integrated into the scaffold and compare this with cellular deformation within the ECM.

ACKNOWLEDGEMENTS

Funded by NIH grants HL-069368 and HL-68816

REFERENCES

1. Sacks, MS et al. J. Biomaterials. 2006 (In press)