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Image-Based Multiscale Structural Models of Fibrous Engineered Tissues

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Abstract—**While it is firmly established that the mechanical behavior of most biological tissues, including bioengineered tissues, is governed by an underlying network of protein fibers, it is still not clear how best to obtain and utilize structural information to predict mechanical response. In this paper, methods are presented to (1) quantify the fiber arrangement in a tissue from different imaging tools, (2) incorporate that structure into a multiscale model, and (3) solve the model equations to predict both the microscopic and the macroscopic tissue response. In principle these concepts could be applied to any tissue (incorporating the specific tissue components as needed), but for demonstration purposes, the focus of the current work is on cell-compacted collagen gel, a model engineered tissue.**

I. Introduction

VERY much work has been done on the mechanical behavior of soft tissues (summarized in [1]), with tremendous advances being made in our understanding of the role of composition and structure in determining the macroscopic properties. Structure, in particular, is commonly handled by methods drawn from the theory of composite materials [2],[3], particularly by invariant-based methods [4] or by methods that integrate over a distribution of fibers. Neither approach, however, accounts directly for fiber-fiber interactions and the resulting non-affinity of the fiber motion. To account for fiber-fiber interactions, a small network of fibers can be analyzed in place of a macroscopic constitutive law, bridging the scale between the fiber network and the tissue via averaging methods [5],[6].

In using such an approach, one must overcome three challenges. First, one must acquire structural information about the tissue – that is, a structural model, regardless of how it is effected mathematically, can be no more accurate than the structural information provided to it. Second, one must translate that information into the specification of the model. This may be simple or difficult depending on the format of the model. Third, one must solved the model equations. In the sections below, we describe each step as implemented for our multiscale structural modeling scheme.

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II. Methods

A. Structural Characterization of Bioengineered Tissues

A major technique for the characterization of collagenous tissues in particular is polarimetric fiber alignment imaging (PFAI), in which polarized light is passed through the tissue and then through a second polarizer rotating during the experiment [7]. The resulting images are processed to determine an equivalent optical element, which provides pixelwise measurement of the retardation and extinction angle for the tissue. In collagen gels, the extinction angle is the direction of primary fiber orientation, and the retardation is related to the degree of alignment. Thus, PFAI allows direct assessment of the alignment state of the tissue sample.

One challenge with PFAI is that it only provides information about alignment in the plane of the tissue. For alignment in the transverse direction, another method is necessary. We use scanning electron microscopy to visualize the fibers in the tissue, subsequently analyzing the SEM image via a Fourier-transform method [8] to determine an approximate fiber orientation distribution. Briefly, if one moves along a fiber, the image intensity changes slowly, giving a low-frequency response, whereas if one moves across fibers, the image intensity changes quickly, giving a high-frequency response. Once the distribution has been generated, the appropriate structural parameters are easily extracted.

B. Construction of Simulated Fiber Networks

In our multiscale scheme, a different fiber network is used at each Gauss point in the finite-element representation of the tissue. Fiber networks are generated by a seed-and-grow technique [9]. Seed points are selected randomly throughout a cubic domain, and a growth direction is selected randomly for each seed. A fiber is then extended from each seed in both directions and continues extending until it intersects another fiber or the edge of the domain. Finally, the domain is truncated to eliminate edge/corner effects [10].

Anisotropy in the fiber network is generated by drawing the fiber growth directions from a non-uniform distribution. Since fibers are of different lengths, and thus contribute unequally to the overall network alignment (which corresponds to what is measured by PLAI), it cannot be certain *a priori* that a given distribution of growth directions will produce the desired final network state. The process was therefore iterated for each network until the extinction direction from the PLAI and the principle alignment

direction from the model were within 8° of each other (dot product greater than 0.99) and the degree of alignment (measured as the eigenvalue of the second-rank orientation tensor for the model network vs. the scaled magnitude of the retardation from PLAI) was within 6%. We have found that statistical homogeneity is achieved for networks containing at least 300 fibers.

C. Model Implementation

The model is implemented by a macroscopic finite-element method coupled to a microscopic network mechanics calculation, as described in detail elsewhere [6]. Briefly, the method relies on the concept of the volume-averaged stress,

$$S_{ij} = \frac{1}{V_\omega}\int_\omega s_{ij}\,dV, \tag{1}$$

where S is the average stress, s is the local stress, and V is the volume of the averaging domain ω. It has been shown [5] that for a materially-deforming averaging volume, the condition of microscopic mechanical equilibrium,

$$s_{ij,i} = 0, \tag{2}$$

implies the following macroscopic balance:

$$S_{ij,i} = \frac{1}{V_\omega}\oint_{\delta\omega}\left(s_{ij} - S_{ij}\right)u_{k,i}n_k\,dA, \tag{3}$$

where $\delta\omega$ is the boundary of ω with unit outward-directed normal n, u is the displacement of the tissue, and index notation is used in both (2) and (3). The extra term, which arises from correlations between the displacement field and the stress field, vanishes if the averaging volume is fixed but not when the averaging volume is deformed.

The macroscopic system (3) is solved as follows. First, an initial guess of the nodal displacements is made. Second, the displacement field is determined in a very small region around each Gauss point and used to specify the boundary deformations of a microscopic network problem. The network mechanics problem is solved by requiring no net force on any interior node (i.e., fiber-fiber intersection). Once the network problem has been solved, the integrals in (1) and (3) are evaluated and passed up to the macroscopic scale. If the weak form of (3) is satisfied, then the problem is done. If not, a Newton iteration is taken for the macroscopic nodal position, and the process repeats.

D. Test Problem: Compacted Collagen Cruciform

The method is generally applicable, but a test problem is obviously necessary. We use the compacted, cell-populated collagen cruciform, which has non-uniform fiber orientation (fibers tend to align along the arms, for example), shows interesting mechanical behavior, and is amenable to biaxial testing with concurrent PLAI.

Samples were prepared using human dermal fibroblasts (10^6 cells/ml) and acid-solubilized type I collagen (Organogenesis, 1.5 mg/ml). The cruciforms had two wide arms (8 mm) and two narrow arms (4 mm), leading to asymmetric alignment. Gels were cultured for four days, which is long enough to produce significant compaction and realignment of the collagen network but short enough that chemical effects (e.g., synthesis and degradation) are negligible.

III. Results

A. Structural Characterization and Network Generation

Figure 1 shows representative PLAI and SEM-based orientation data images, which were used to construct networks such as that shown in Figure 1d.

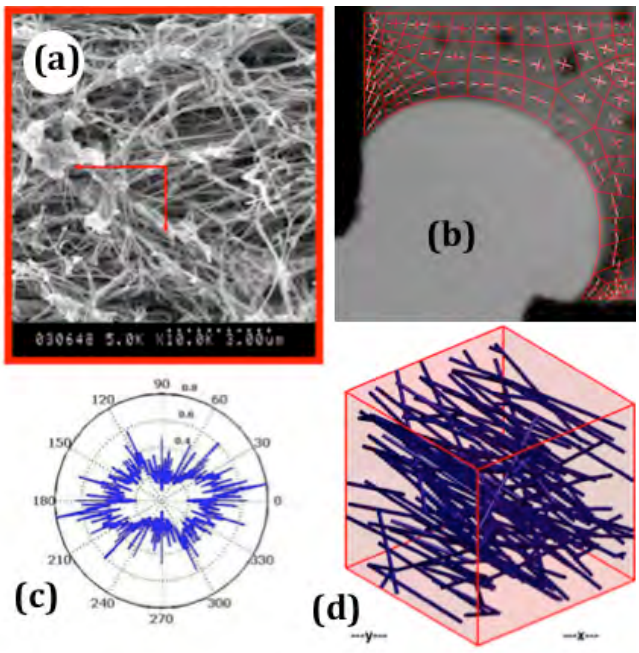

Fig. 1. (a) Scanning electron micrograph of a transverse slice of a compacted collagen network. (b) PLAI image of one quarter of cruciform, with red vectors showing measured fiber orientation and white vectors showing model representation. (c) Fiber alignment as calculated via the Fourier transform method applied to image (a). (d) Typical model network. This network is strongly aligned with the y axis.

B. Mechanical Testing

Two mechanical tests were performed. First, strip biaxial (off-axis hold) tests were performed, in which the sample was extended vertically and held at constant length horizontally. The experiment was simulated using the model, and two constants for the fiber mechanical model were regressed. The resulting fit for the fiber force F_F was

$$F_f = (14\text{nN})\left[\exp(3.8\varepsilon) - 1\right] \tag{4}$$

where ε is the fiber strain. These two parameters were then used to model an equibiaxial extension experiment (simultaneous stretch in both directions). As can be seen in Figure 2, the model based on the strip biaxial test predicted the equibiaxial test results very well.

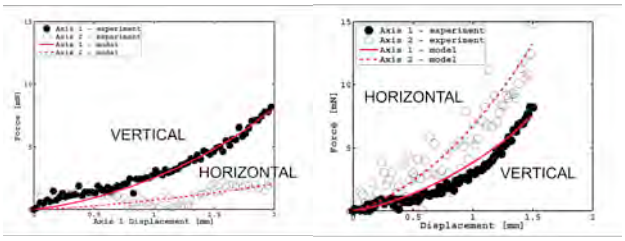

Fig. 2. Mechanical tests of cruciforms. On the left, a strip biaxial test is shown. Since the vertical direction is being extended, the vertical-direction forces are higher than the horizontal. Model fit is shown by the red lines, as described in the text. On the right, an equibiaxial test is shown. In this case, the wider horizontal arms generate larger forces. Importantly, the model (red lines), which used parameters entirely determined by the first test, predicted the second test results well.

IV. Discussion

The image-based multiscale scheme was extremely successful in analyzing and predicting behavior of the four-day compacted collagen gel. The rearrangement of fibers during loading and the mechanical consequences thereof were predicted accurately for one test based on results from another. The method, although requiring multiple steps and considerable computational resources, is quite promising.

There remain, however, significant issues to address. Most importantly, there was only one component – collagen – in the gel, and the cells were quite dilute and thus not expected to contribute a large amount to the mechanical properties of the gel. In a real tissue, be it native or engineered, there is a high degree of cellularity, as well as a wide range of other structural proteins besides collagen (e.g., fibrin or elastin) and proteoglycan. In principle, our multiscale scheme should be well positioned to address the mechanical roles of the different components, but implementation of such a detailed model will be far from trivial. The challenge will be particularly great because the hierarchical nature of many tissue architectures may require additional levels beyond the simple two-scale model presented herein.

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References

[1] Y. C. Fung, *Biomechanics: Mechanical Properties of Living Tissues.* New York: Springer-Verlag, 1993, pp. 568.

[2] M. S. Sacks, "Incorporation of experimentally-derived fiber orientation into a structural constitutive model for planar-collagenous tissues," *Journal of Biomechanical Engineering-Transactions of the Asme,* vol. 125, pp. 280-287, Apr. 2003.

[3] Y. Lanir, "Constitutive equations for fibrous connective tissues," *J. Biomech.,* vol. 16, pp. 1-12, 1983.

[4] A. D. Freed, D. R. Einstein and I. Vesely, "Invariant formulation for dispersed transverse isotropy in aortic heart valves: an efficient means for modeling fiber splay," *Biomech. Model. Mechanobiol,* vol. 4, pp. 100-117, Nov. 2005.

[5] T. Stylianopoulos and V. H. Barocas, "Volume-averaging theory for the study of the mechanics of collagen networks," *Comput. Methods Appl. Mech. Eng.,* vol. 196, pp. 2981-2990, 2007.

[6] P. L. Chandran, T. Stylianopoulos and V. H. Barocas, "Microstructure-based, multiscale modeling for the mechanical behavior of hydrated fiber networks," *SIAM MMS,* vol. 7, pp. 22-43, 2008.

[7] T. T. Tower, M. R. Neidert and R. T. Tranquillo, "Fiber alignment imaging during mechanical testing of soft tissues," *Ann. Biomed. Eng.,* vol. 30, pp. 1221-1233, 2002.

[8] E. A. Sander and V. H. Barocas, "Comparison of 2D fiber network orientation measurement methods," *J. Biomed. Mater. Res. A.,* Feb 19. 2008.

[9] P. L. Chandran and V. H. Barocas, "Affine vs. non-affine fibril kinematics in collagen networks: theoretical studies of network behavior," *J. Biomech. Eng.,* vol. 128, pp. 259-270, 2006.

[10] D. S. Clague and R. J. Phillips, "A numerical calculation of the hydraulic permeability of three-dimensional disordered fibrous media," *Phys. Fluids,* vol. 9, pp. 1562, 06. 1997.