SI Appendix

Overview of the Multi-scale Approach.

Our multi-scale methodology is based on the fundamental assumption that the tissue mechanical behavior is too complex to be modeled with a continuum level constitutive equation. Thus, in order to relate the macroscopic deformation of the tissue to the macroscopic stress, we introduce a microscopic scale, which consists of location-matched collagen networks, also called representative volume elements (RVEs), that provides a statistical representation of the local tissue microstructure. The RVE represents the collagen microstructure as a three-dimensional network of interconnected fibers. The connections between fibers (fiber-fiber interactions) are represented as rigid, freely rotating cross-links, and the mechanical response of the individual fiber is governed by a fiber constitutive equation. At the same time, we model the macroscopic scale with a Galerkin finite element method.

The RVEs are constructed around each integration point of the finite element model, and the boundary displacements are interpolated through the element basis functions from the macroscopic deformation field. Boundary-connected fibers develop forces that are then transmitted into the network. A force balance among the fibers determines the equilibrium network state, and the volume-averaged Cauchy stress is calculated for use on the macroscopic scale. The averaged stress balance is solved at the macroscopic level to determine the macroscopic displacement field, and this process continues to iterate until the solution converges. Thus, by solving a set of microstructural problems simultaneously, the tissue-level behavior of the collagen can be related directly to the fiber mechanics and the network structure (Fig. S1). Below we present the details of our multi-scale model.

3-D Microscopic Collagen Network (RVE) Formation

3-D fiber networks are created with a custom MATLAB routine based on specification of fiber number, PFAI strength of alignment (eigenvalues of the orientation tensor), and PFAI preferred direction (eigenvectors of the orientation tensor). First, a number of seed points are generated and distributed randomly (from a uniform distribution) inside a box. Next, a seed point is selected randomly and grown a segment length along its direction vector in either the positive or negative direction. If growth extends the fiber beyond the boundary of the box, that end of the fiber is repositioned at the boundary and removed from the pool of growing fiber ends. Alternatively, if the fiber contacts another fiber, the fibers are joined via a cross-link, and growth of that fiber end is terminated. This process continued until there are no more growing fibers. After the network is formed, a smaller network is extracted from the center of the box. Fibers in the network are defined as segments between two nodes, where a node either forms a termination point on the boundary or a cross-link between fibers. Cross-links are modeled as pin joints – free to rotate but unable to slip. As detailed in the Methods section of the paper, networks are created for each element so that their projected 3-D structure match within a tolerance the average direction and strength of alignment (assessed by PFAI) in the corresponding location of the cruciform.

Volume-averaged Cauchy Stress Tensor

The macroscopic, volume-averaged Cauchy stress tensor, S_{ij} is given by:

$$S_{ij} = \frac{1}{V} \int_V s_{ij} dV, \qquad \text{(S-1)}$$

where V is the volume of the RVE and s_{ij} is the microscopic stress tensor of the RVE. Note that throughout this derivation we use index notation. The gradient of the directional vector **x** can be written as

$$x_{i,j} = \delta_{ij}, \qquad \text{(S-2)}$$

where δ_{ij} is the Kronecker delta. Thus, the microscopic stress tensor can be written as

$$s_{ij} = s_{kj}\delta_{ik} = s_{kj}x_{i,k}, \qquad \text{(S-3)}$$

and (S-1) can be rewritten as

$$S_{ij} = \frac{1}{V}\int_V \left(s_{kj}x_i\right)_{,k} dV - \frac{1}{V}\int_V s_{kj,k}x_i dV. \quad \text{(S-4)}$$

Application of the divergence theorem and microscopic equilibrium, $s_{kj,k} = 0$, gives

$$S_{ij} = \frac{1}{V}\oint_{\partial V} n_k s_{kj} x_i dA = \frac{1}{V}\oint_{\partial V} x_i t_j dA \qquad \text{(S-5)}$$

where the dot product $n_k s_{kj}$ is the traction, t_j, exerted on the boundaries of the RVE. For a network of thin fibers, each boundary cross-link may be treated as a point with the integral of the traction being the force on the cross-link, so Eq. (S-5) can be written as [1]:

$$S_{ij} = \frac{1}{V}\sum_{boundary\ nodes} x_i f_j \qquad \text{(S-6)}$$

where x is the position of the boundary cross-link and f is the force, defined by the fiber constitutive equation.

Because the RVE is dimensionless, the dimensionless RVE volume V and position x_i in Eq. (S-6) must be converted to dimensional quantities that represent a specific space in the tissue. The RVE represents a cube in the tissue of edge length α, the collagen fiber volume fraction is θ, and the fiber cross-sectional area is A_f. If in the dimensionless RVE scale the total fiber length is L (dimensionless quantity), then the total fiber length in the tissue-level is αL, and the fiber volume is $A_f \alpha L$. Therefore the fiber volume fraction and the value of α are given by

$$\theta = \frac{A_f L\alpha}{\alpha^3} = \frac{LA_f}{\alpha^2} \Rightarrow \alpha = \sqrt{\frac{LA_f}{\theta}} \quad \text{(S-7)}$$

and Eq. (S-6) is rewritten as [2]:

$$S_{ij} = \frac{1}{V'}\sum x'_i f_j \Rightarrow S_{ij} = \frac{\theta}{LA_f}\frac{1}{V}\sum_{boundary\ crosslinks} x_i f_j\,, \quad \text{(S-8)}$$

where the prime denotes dimensional quantity, and we have to consider that $V' = V \cdot \alpha^3$ and $x'_i = x_i \cdot \alpha$.

Governing Equations

The formulation described above requires a fiber-level constitutive equation for collagen and the appropriate form of the Cauchy stress balance. The mechanical behavior of individual fibers is governed by a phenomenological equation [2, 3]:

$$f = \frac{E_f A_f}{B}\left[\exp(B \cdot 0.5 \cdot (\lambda_f^2 - 1) - 1\right], \qquad \text{(S-9)}$$

where f is the force generated, E_f is the small-strain fiber modulus, A_f is the fiber cross-sectional area, λ_f is the fiber longitudinal stretch ratio, and the constant β captures the non-linearity of the response; in the limit $\lambda_f \to 1$, Eq. (S-9) reduces to a linear elastic fiber with modulus E_f.

A detailed derivation of the macroscopic force balance can be found in [4]. Microscopic equilibrium is assumed, i.e., the divergence of the microscopic stress tensor is zero ($s_{ij,i} = 0$), and using Leibnitz theorem, the following expression for the divergence of the macroscopic stress tensor is derived:

$$S_{ij,i} = \frac{1}{V}\oint_{\partial V}\left(s_{ij} - S_{ij}\right)u_{k,i} n_k dA, \quad \text{(S-10)}$$

where u_k is the displacement of the RVE boundary, and n_k is the unit normal vector. The right hand side of Eq. (S-10) is due to coupling between the non-uniform stress and the non-uniform deformation of the RVE boundary.

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