Viscoelastic and Growth Mechanics in Engineered and Native Tendons

S.C. Calve[1], H. Narayanan[2], K. Garikipati[2], K. Grosh[2,3] and E.M. Arruda[1,2]

[1]Macromolecular Science and Engineering

[2]Mechanical Engineering

[3]Biomedical Engineering

The University of Michigan

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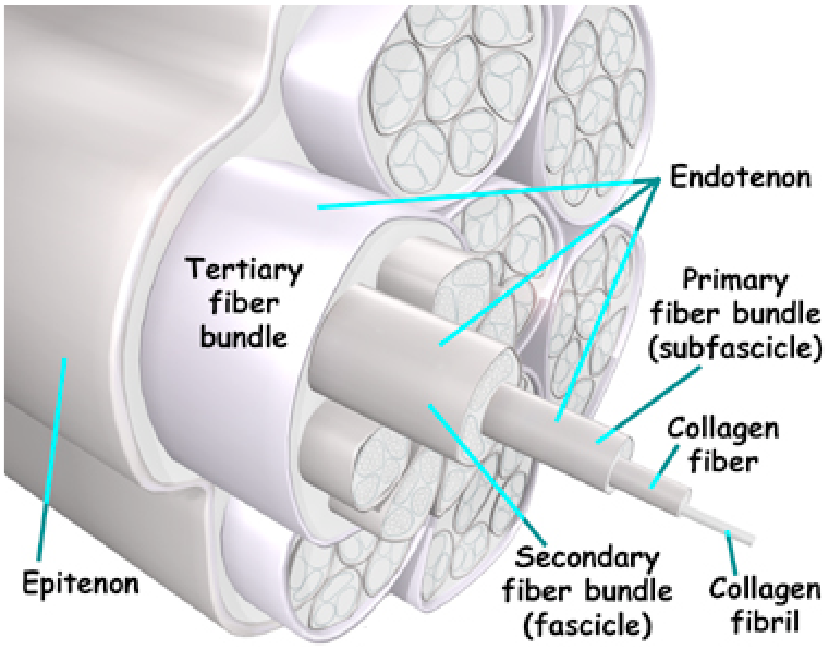
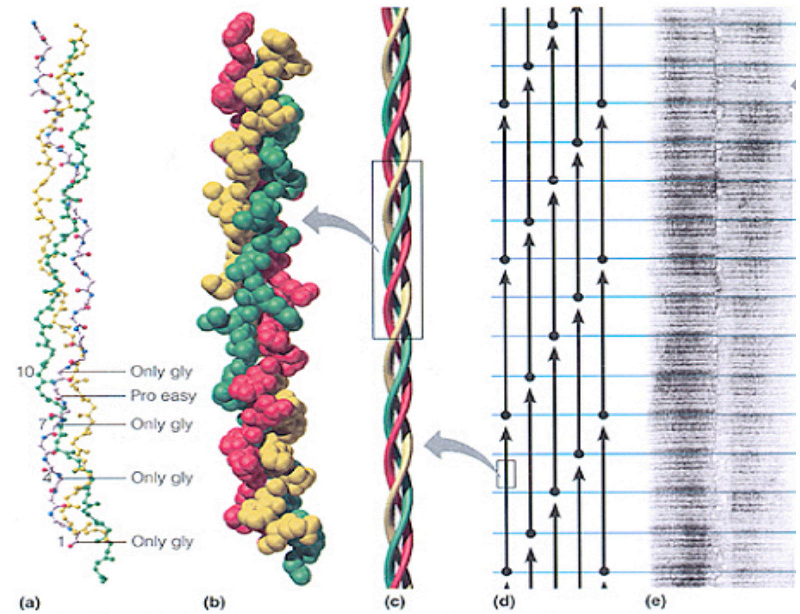
Motivation

- To characterize and develop mathematical models for the evolution of mechanical properties during the growth of collagen-based native tissues
- To engineer functional, implantable collagen-based tissue constructs in vitro, for studies of growth both in vitro and in vivo

(Collagen-Based) Soft Tissue Model: Tendon





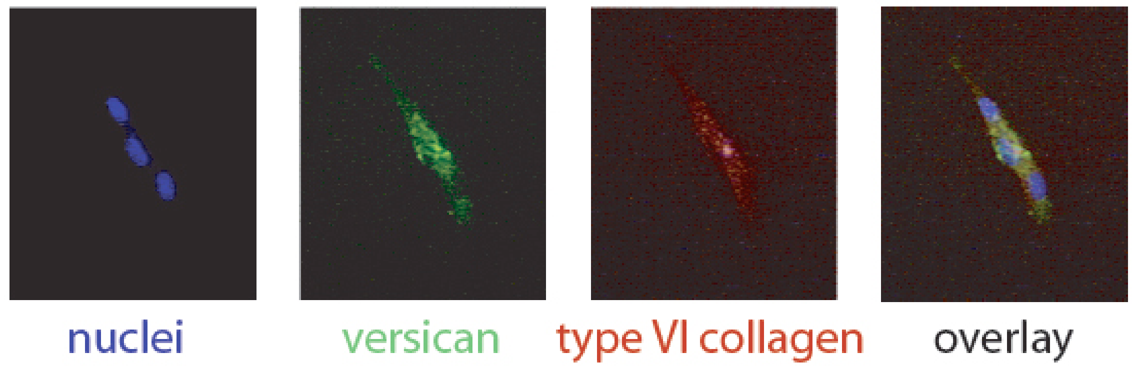
Adult tendon

- Relatively avascular
- Relatively acellular
- Non-innervated
- 80% of dry weight is type I collagen

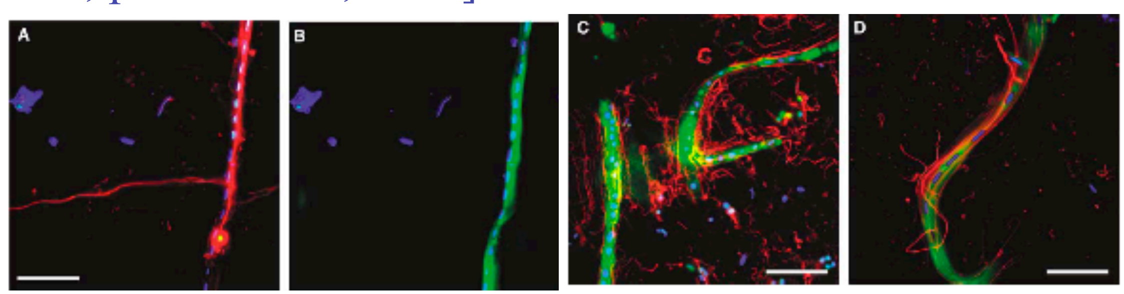
Tissue Engineering: Tendon Cells Deposit a Physiologically Relevant Matrix In-Vitro

- Why in vitro models? Physiological relevance?
- Fisher F344 rat tendon cells are plated on natural mouse laminin coated substrates, in media supplemented with growth factors
- The cells form tendon cell arrays, secrete and organize a pericellular environment similar to that found in vivo within 48 hours of plating: versican and type VI collagen

Rat tendon cell arrays engineered in-vitro [Calve et al.]



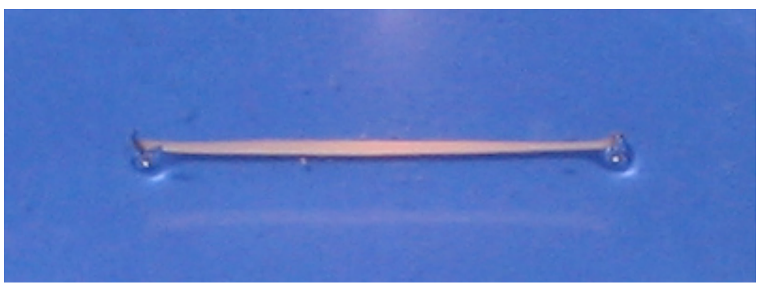
Canine tendon cell arrays in-vivo [Ritty et al., Structure, V11, p1179-1188, 2003]

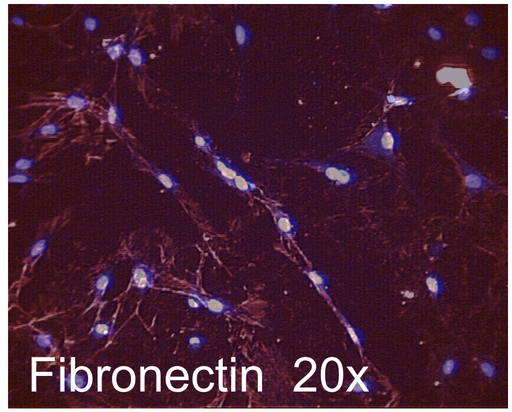


A fibrillin-2 (red) [bar 80 mm], B versican (green), C and D fibrillin and versican [bar 120 mm in C and 80 mm in D]

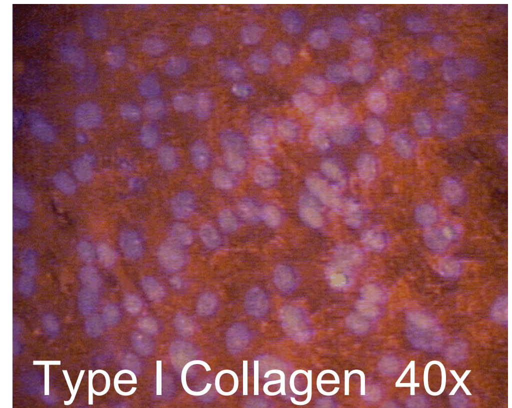
Tendon Engineering by the Self-Organization of Cells and their Autogenous Matrix In-Vitro

- Cells continue to express proteins associated with the ECM in culture
- After approximately 2 weeks in culture the cells and ECM lift off the substrate and contract into a cylindrical construct
- Homogeneous, 12 mm long

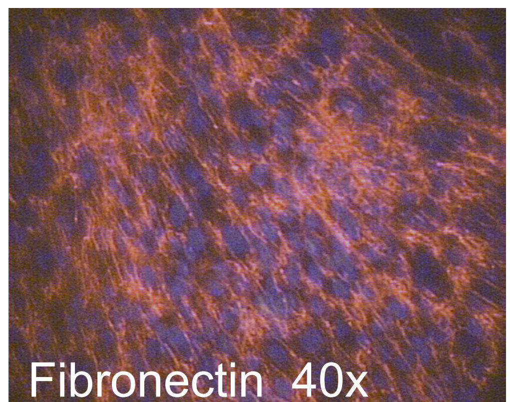




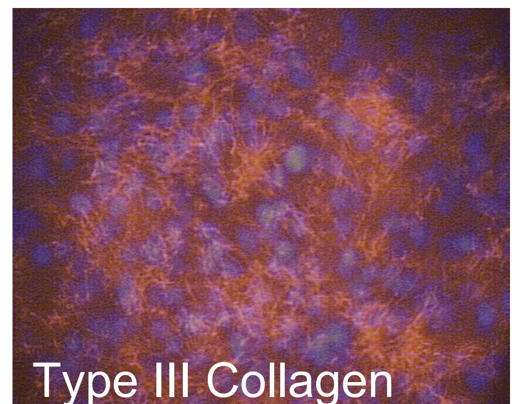
Fibronectin 20x
1 day



Type I Collagen 40x
10 days



Fibronectin 40x
10 days



Type III Collagen
10 days

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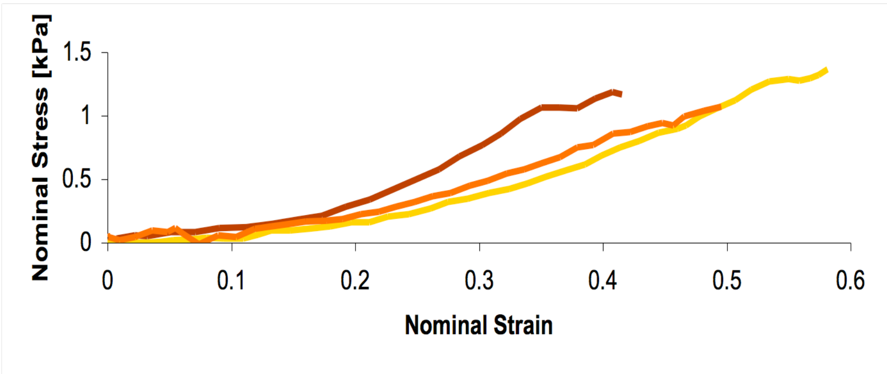
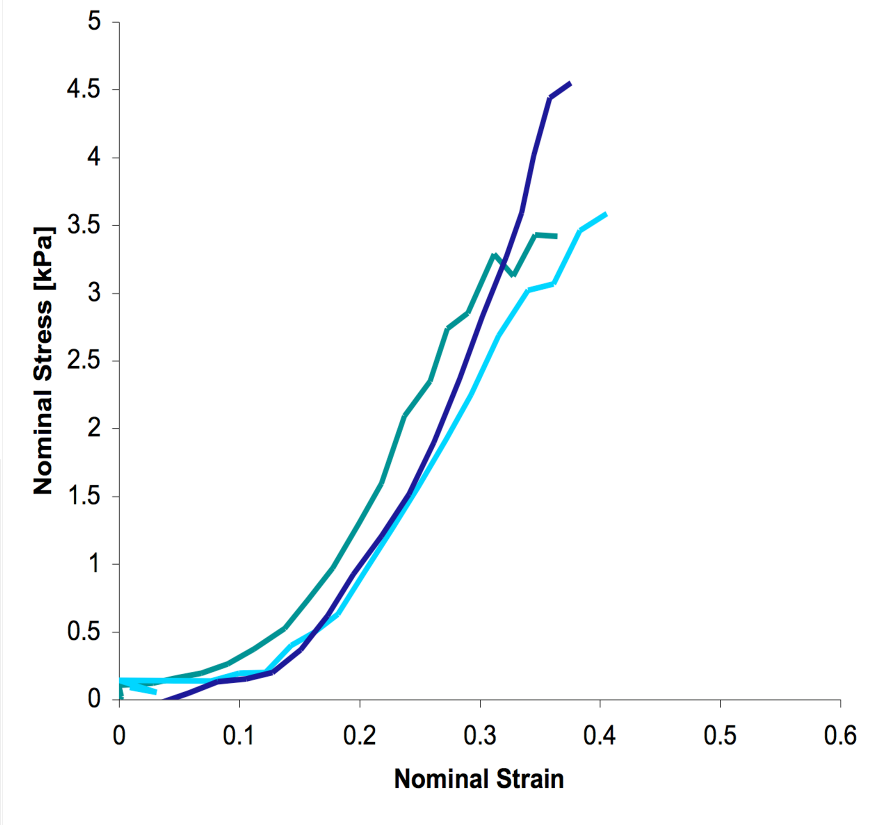
Homogeneous Growth in Engineered Constructs

As-formed (0.01/sec)



Four weeks in static culture (0.01/sec)



Both an increase in collagen content and cross-linking play a role

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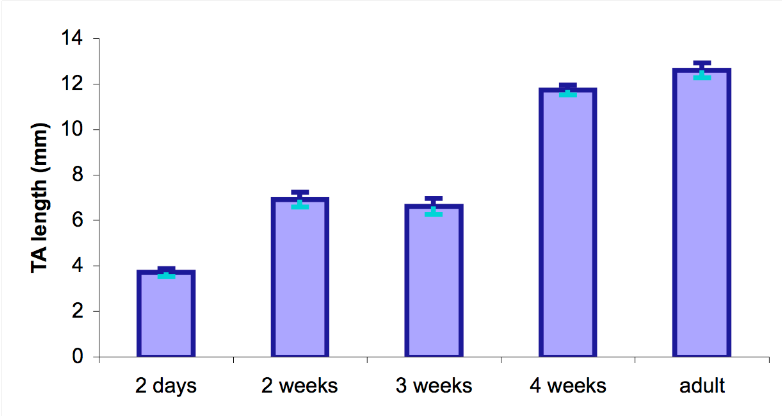
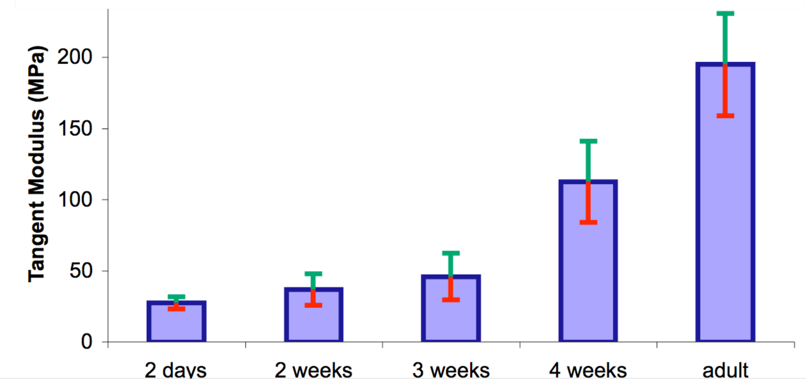
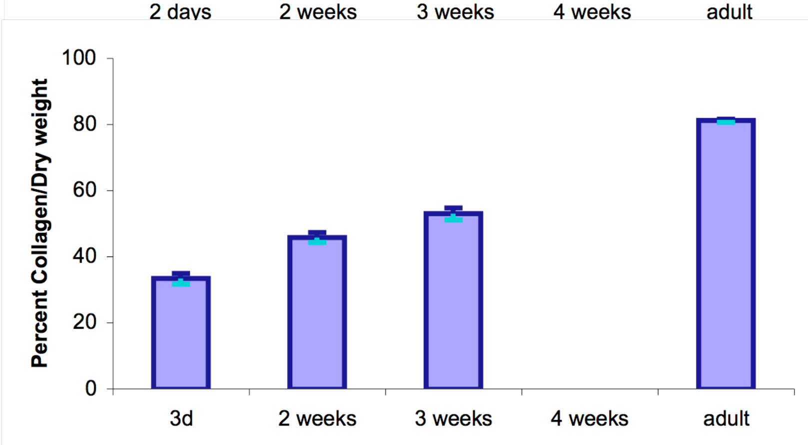
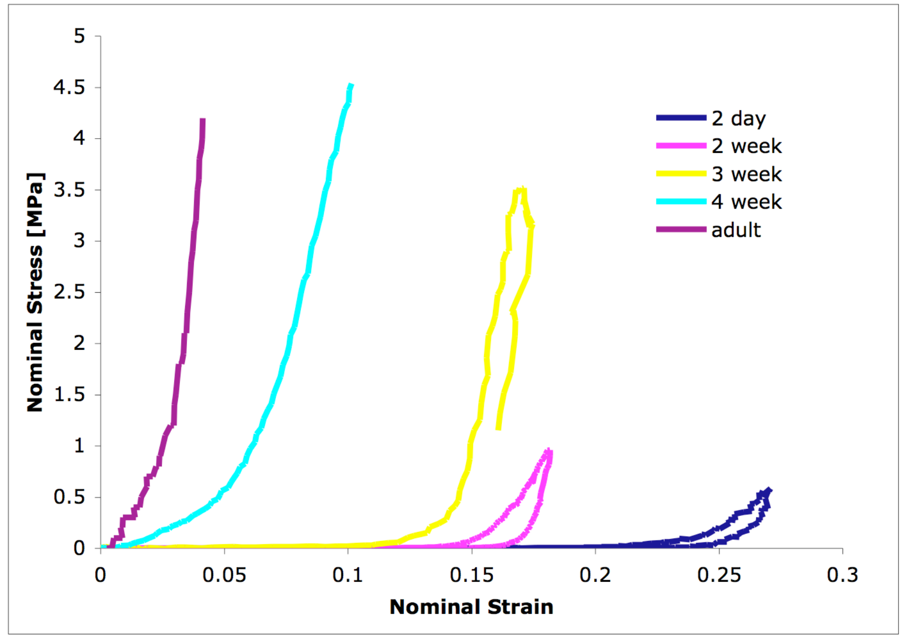
Growth of Rat Tibialis Anterior Tendon









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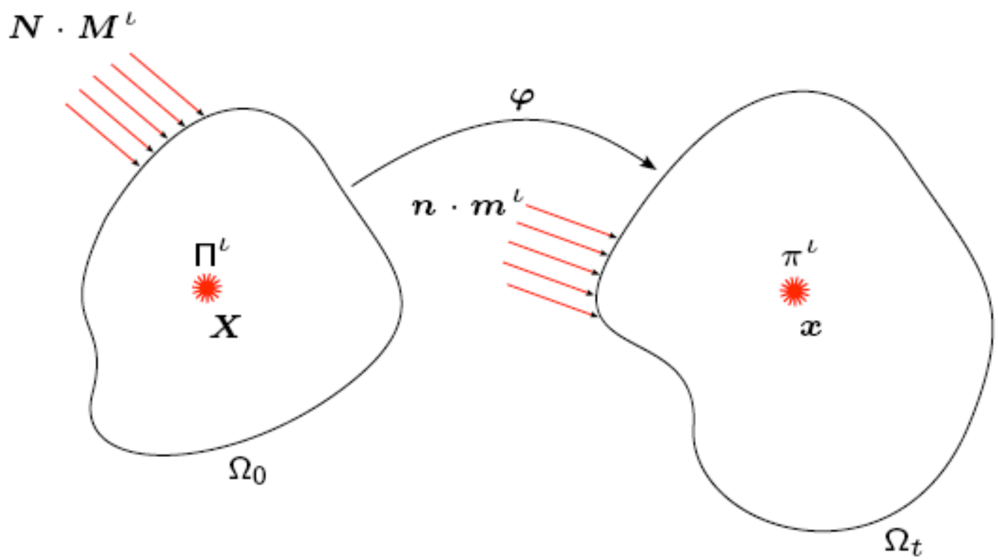
Modelling Approach

- Growth: An addition of mass to the tissue
- Classical balance laws enhanced via fluxes and sources
- Multiple species inter-converting and interacting:
  - Solid: Collagen, proteoglycans, cells
  - Extra cellular fluid: Water (undergoes transport relative to the solid)
  - Dissolved solutes: Sugars, proteins, … (undergo transport relative to fluid)

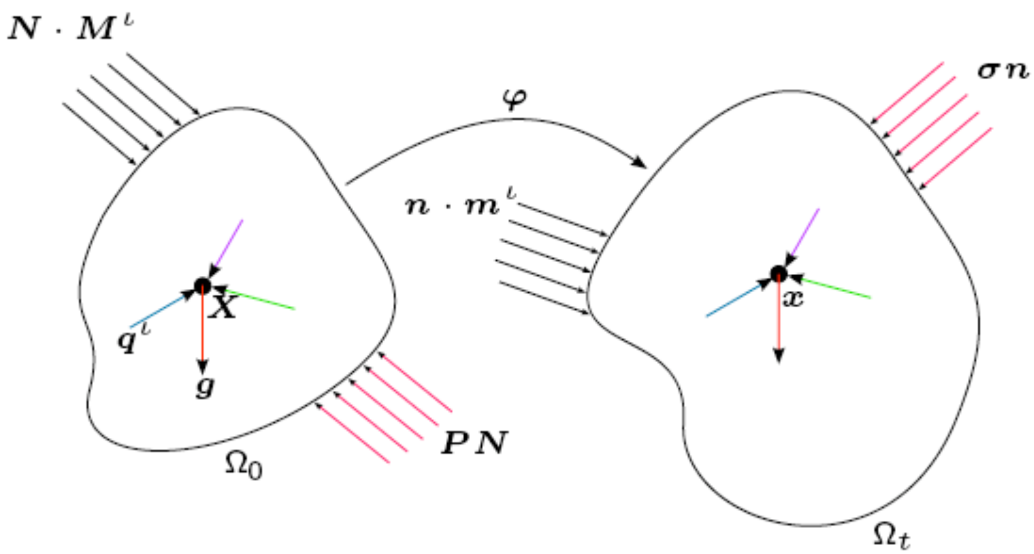
Mass Balance



$$\frac{\partial \rho_0^{\iota}}{\partial t} = \Pi^{\iota} - \boldsymbol{\nabla}_X \cdot \boldsymbol{M}^{\iota}$$

ρ_0^{ι} – species concentration
Π^{ι} – species production
$\boldsymbol{M}^{\iota}$ – species flux

Momentum Balance



ρ_0^ι – species concentration
$\boldsymbol{V}$ – solid velocity
$\boldsymbol{V}^\iota$ – species relative velocity
$\boldsymbol{g}$ – body force
$\boldsymbol{q}^\iota$ – interaction force
$\boldsymbol{P}^\iota$ – partial stress

$$\rho_0^\iota \frac{\partial}{\partial t}\left(\boldsymbol{V} + \boldsymbol{V}^\iota\right) = \rho_0^\iota\left(\boldsymbol{g} + \boldsymbol{q}^\iota\right) + \boldsymbol{\nabla}_X \cdot \boldsymbol{P}^\iota - \left(\boldsymbol{\nabla}_X\left(\boldsymbol{V} + \boldsymbol{V}^\iota\right)\right)\boldsymbol{M}^\iota$$

Constitutive Framework

- Consistent with the dissipation inequality
- Constitutive hypothesis: $e^{\iota} = \hat{e}^{\iota}(\mathbf{F}^{e^{\iota}}, \rho_0^{\iota}, \eta^{\iota})$
- Collagen Stress: $\mathbf{P}^c = \rho_0^c \frac{\partial e^c}{\partial \mathbf{F}^{e^c}} \mathbf{F}^{g^c -T}$
  - Hyperelastic Material
  - Continuum stored energy function based on the Worm-like chain model
- Fluid Stress: $\mathbf{P}^f = \rho_0^f \frac{\partial e^f}{\partial \mathbf{F}^{e^f}} \mathbf{F}^{g^f -T}$
  - Ideal Fluid
  - $\rho_0^f \hat{e}^f = \frac{1}{2}\kappa(det(\mathbf{F}^{e^f}) - \mathbf{1})^2$, κ – fluid bulk modulus
- Fluid flux relative to collagen

$$\mathbf{M}^f = \mathbf{D}^f \left(\rho_0^f \mathbf{F}^{\mathrm{T}} \mathbf{g} + \mathbf{F}^{\mathrm{T}} \nabla_X \cdot \mathbf{P}^f - \nabla_X (e^f - \theta \eta^f)\right)$$

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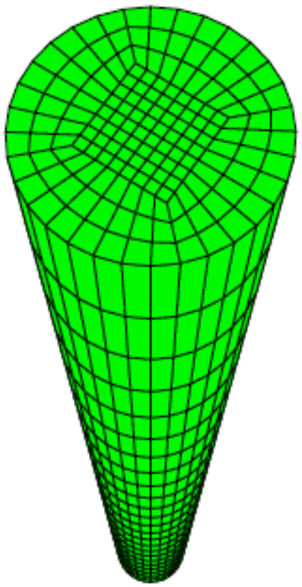
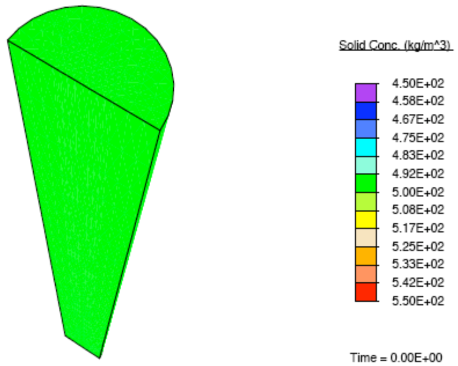
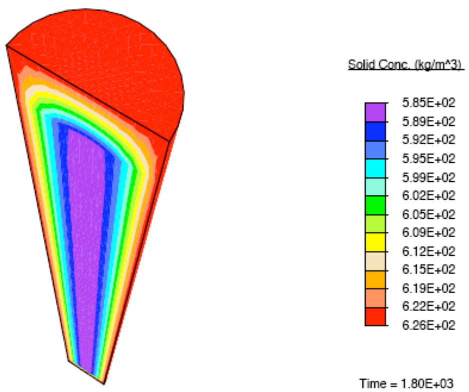
Example: Growth in a Bath

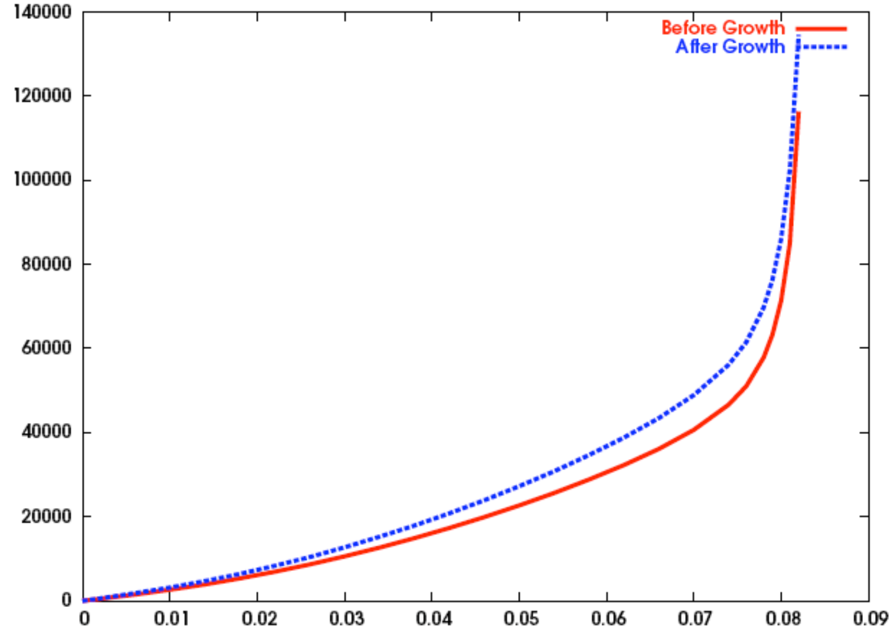


- Biphasic model
  - worm-like chain model for collagen
  - ideal, nearly incompressible interstitial fluid with bulk compressibility of water
  - fluid mobility $D^f_{ij} = 1 \times 10^{-8}\delta_{ij}$, Han et al. [2000]
- "Artificial" sources: $\Pi^{\mathrm{f}} = -k^{\mathrm{f}}(\rho^{\mathrm{f}}_0 - \rho^{\mathrm{f}}_{0_{\mathrm{ini}}}), \quad \Pi^{\mathrm{c}} = -\Pi^{\mathrm{f}}$
- Entropy of mixing: $\eta^f_{\mathrm{mix}} = -\frac{k}{\mathcal{M}^f} \log \frac{\rho^f_0}{\rho_0}$

Example: Growth in a Bath







Stress (Pa) vs Extension (m)

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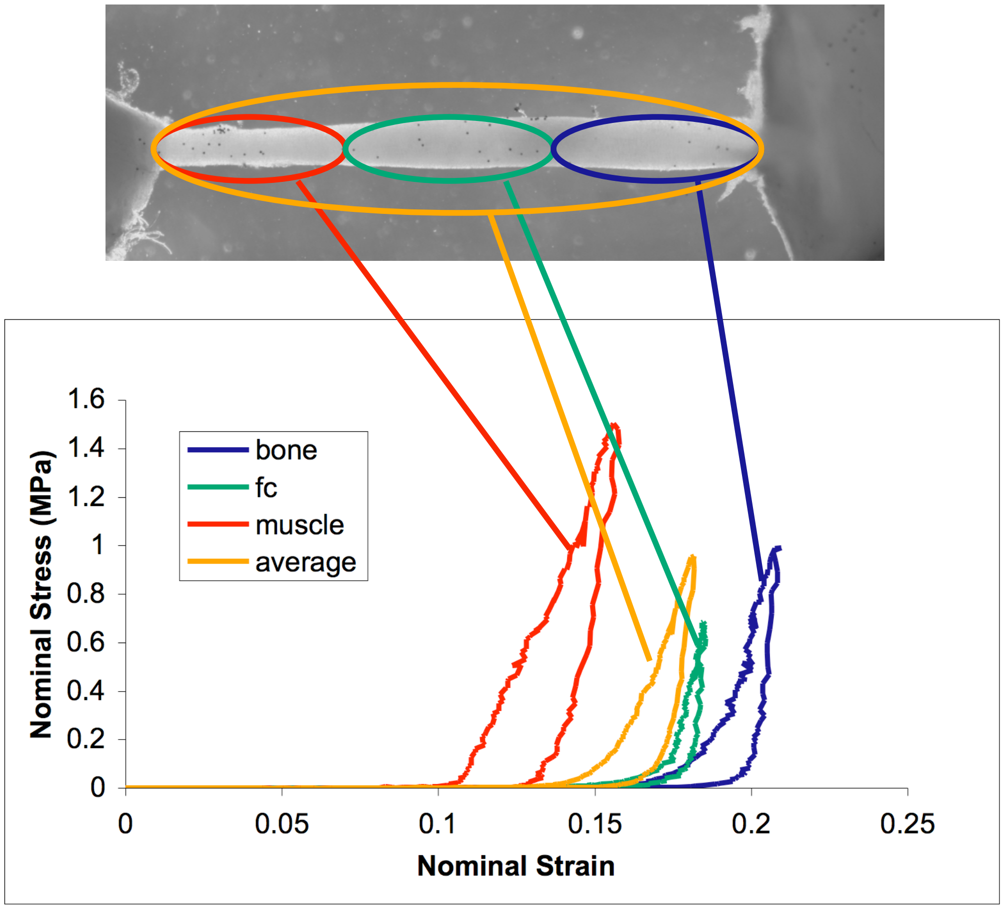
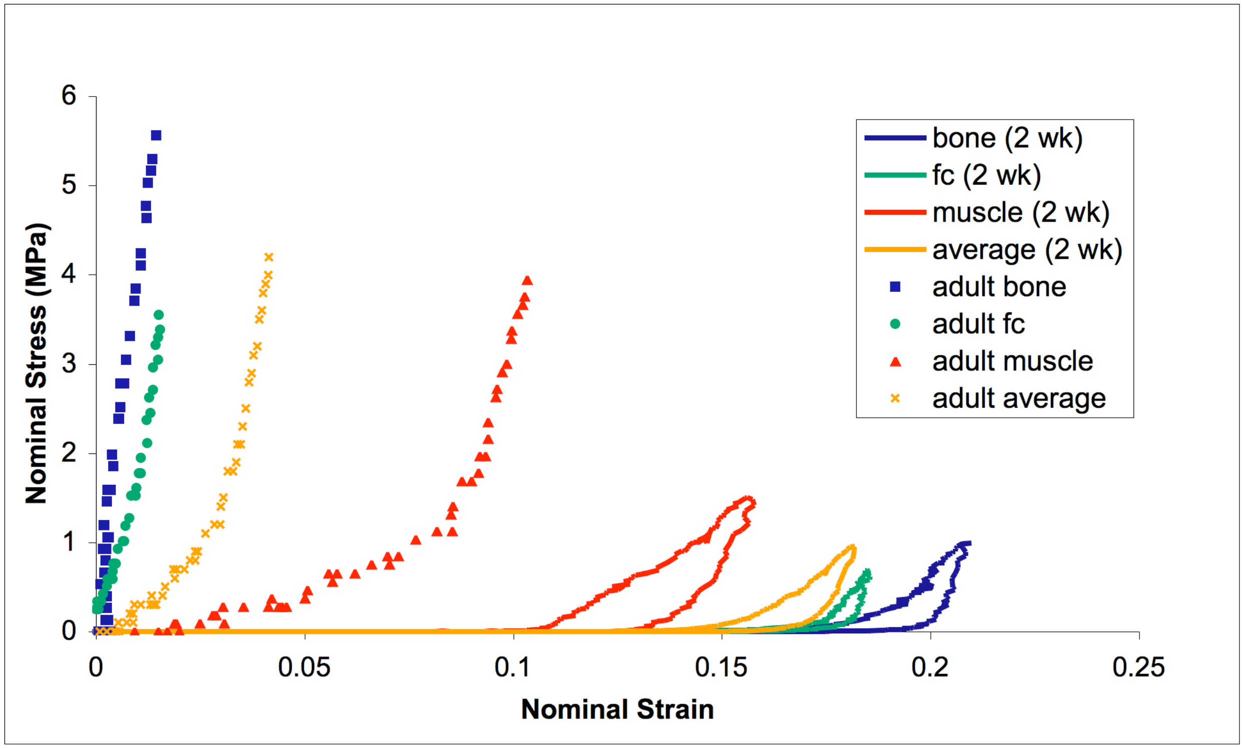
Native Tendon is Functionally Graded



Two week old TA tendon

Tendon Growth is Not Homogeneous



How could this be modelled?

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Choices for Volumetric Sources

- Simple first order rate law – Constituents either "solid" or "fluid"

$$\Pi^{\mathrm{f}} = -k^{\mathrm{f}}(\rho^{\mathrm{f}} - \rho^{\mathrm{f}}_{\mathrm{ini}}), \quad \Pi^{\mathrm{c}} = -\Pi^{\mathrm{f}}$$

- Strain Energy Dependencies – Weighted by relative densities

$$\Pi^{\mathrm{c}} = \left(\frac{\rho^{\mathrm{c}}}{\rho^{\mathrm{c}}_{0_{\mathrm{ini}}}}\right)^{-m}\Psi_0 - \Psi_0^*$$

[Harrigan & Hamilton, 1993]

- Enzyme Kinetics – Introducing additional species to the mixture

$$\Pi^{\mathrm{s}} = \frac{(\Pi^{\mathrm{s}}_{\mathrm{max}}\rho^{\mathrm{s}})}{(\rho^{\mathrm{s}}_m + \rho^{\mathrm{s}})}\rho_{\mathrm{cell}}, \quad \Pi^{\mathrm{c}} = -\Pi^{\mathrm{s}}$$

[Michaelis & Menten, 1913]

- Cell Signalling – Preferential growth in damaged regions

$$\widetilde{\Pi^{\mathrm{c}}} = \alpha\, \Pi^{\mathrm{c}}$$

Enzyme Kinetics

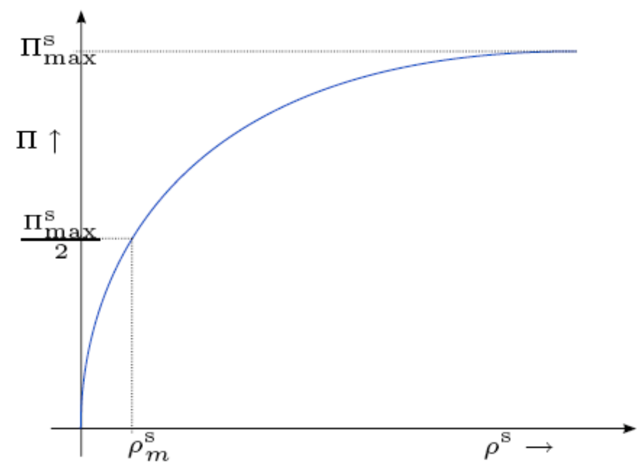
$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

k_1 - Association of substrate and enzyme

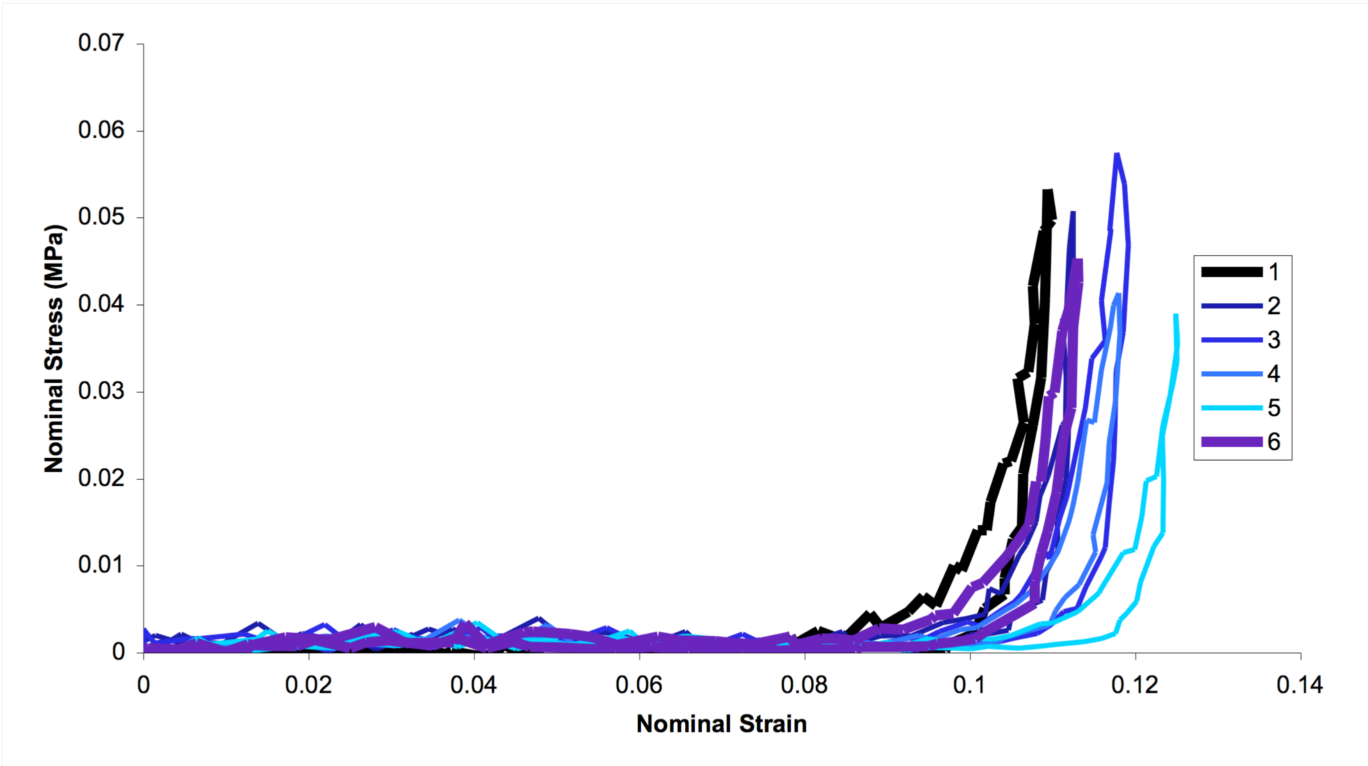
k_{-1} - Dissociation of unaltered substrate

k_2 - Formation of product

$$\rho^{\mathrm{s}}_m = \frac{(k_2 + k_{-1})}{k_1}$$

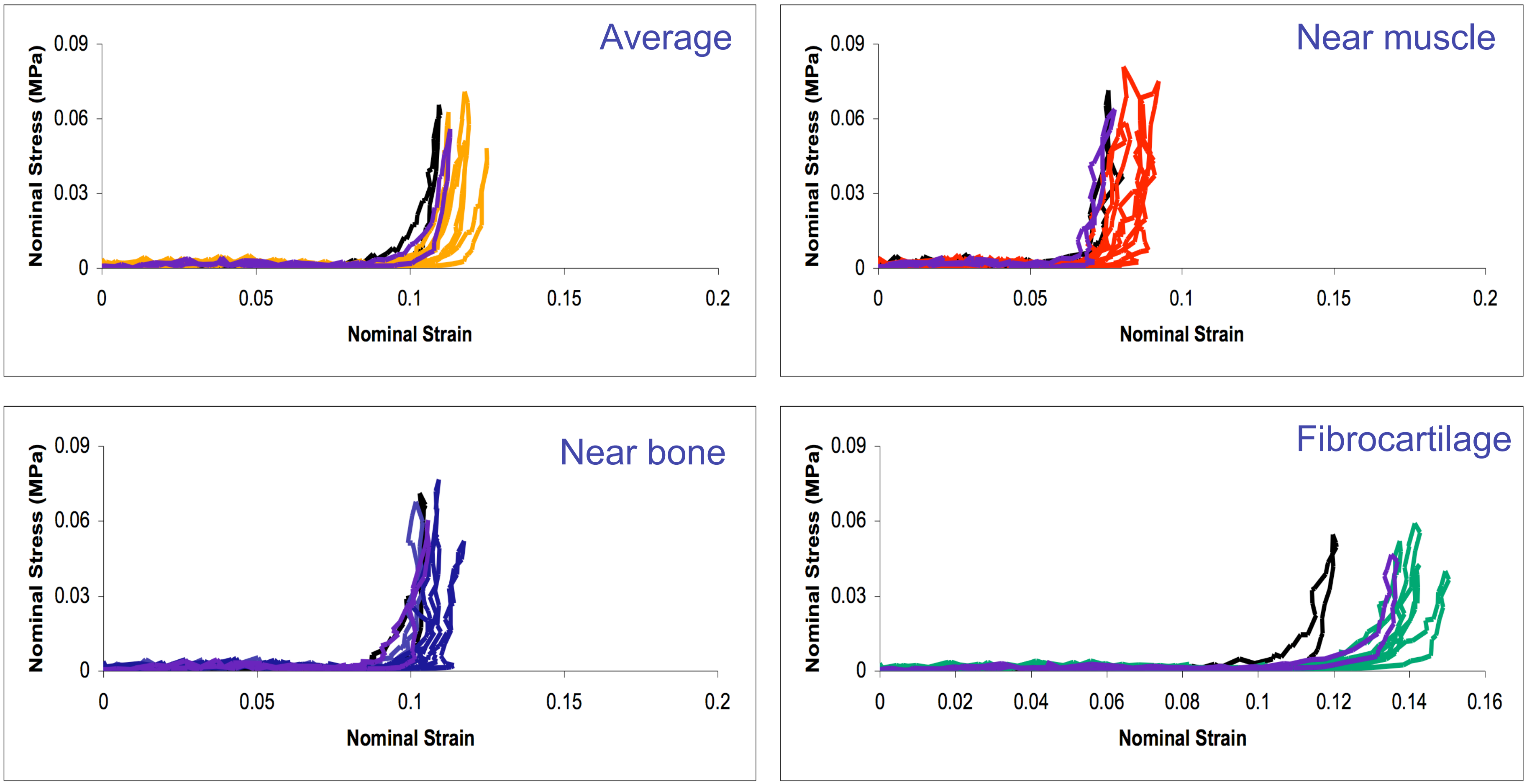


Viscoelastic Response of TA Tendon



Five continuous cycles, 0.01/s, 20 s delay
10 Minute recovery, Sixth cycle at 0.01/s

Regional Variation Manifested in Viscoelastic Response of TA Tendon



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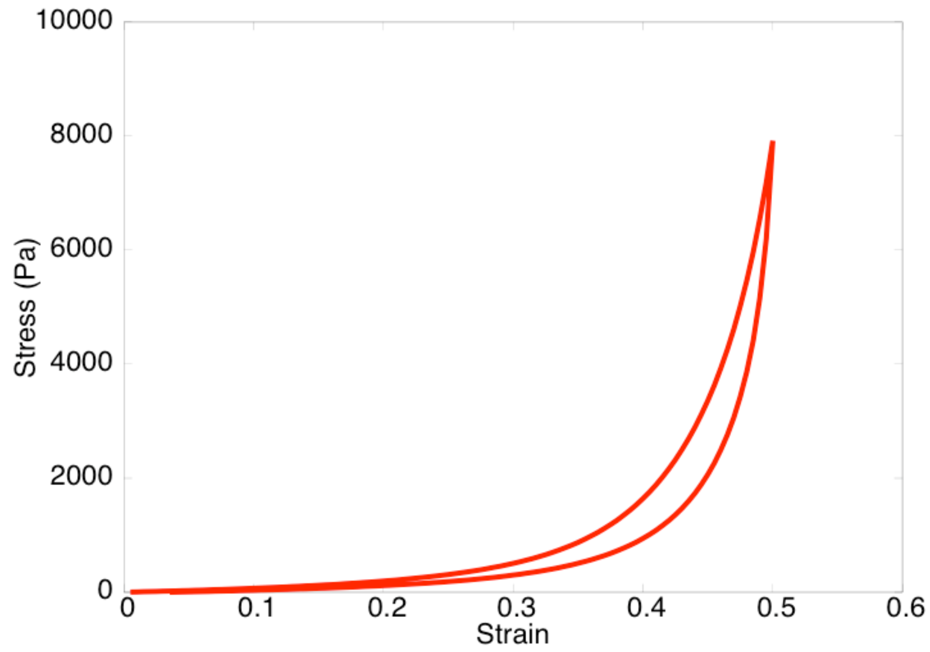
Example: Viscoelasticity

- Tendon immersed in a bath; no growth.
- Strain rate = 0.01/s
- Terms in dissipation inequality result in loss
  - Scaled by mobilities, which are fixed from literature



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Summary and future work

- Highlighted some recent experimental results pertinent to the mechanics of growing tendon
  - Heterogeneity and functional gradation
- Brief introduction to the formulation and modelling choices
- Open issues involving choices for modelling more complex behaviour

- Continue engineering and characterization of growing, functional biological tissue to drive and validate modelling
- Revisit fundamental kinematics assumptions to enhance the model