Viscoelastic and Growth Mechanics in Engineered and Native Tendons

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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
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
Homogeneous Growth in Engineered Constructs

As-formed (0.01/sec) vs. Four weeks in static culture (0.01/sec)

Both an increase in collagen content and cross-linking play a role.
Growth of Rat Tibialis Anterior Tendon

- **TA length (mm):**
  - 2 days
  - 2 weeks
  - 3 weeks
  - 4 weeks
  - Adult

- **Tangent Modulus (MPa):**
  - 2 days
  - 2 weeks
  - 3 weeks
  - 4 weeks
  - Adult

- **Percent Collagen/Dry weight:**
  - 3d
  - 2 weeks
  - 3 weeks
  - 4 weeks
  - Adult

- **Nominal Stress [MPa] vs Nominal Strain:**
  - 2 day
  - 2 week
  - 3 week
  - 4 week
  - Adult

[Graphs showing growth metrics over time]
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

\[ \frac{\partial \rho^t_0}{\partial t} = \Pi^t - \nabla_X \cdot M^t \]

- \( \rho^t_0 \) – species concentration
- \( \Pi^t \) – species production
- \( M^t \) – species flux
Momentum Balance

\[ \rho_0^t \frac{\partial}{\partial t} (\mathbf{V} + \mathbf{V}^t) = \rho_0^t (\mathbf{g} + \mathbf{q}^t) + \nabla_X \cdot \mathbf{P}^t - (\nabla_X (\mathbf{V} + \mathbf{V}^t)) \mathbf{M}^t \]

- \( \rho_0^t \) – species concentration
- \( \mathbf{V} \) – solid velocity
- \( \mathbf{V}^t \) – species relative velocity
- \( \mathbf{g} \) – body force
- \( \mathbf{q}^t \) – interaction force
- \( \mathbf{P}^t \) – partial stress
Constitutive Framework

- Consistent with the dissipation inequality
- Constitutive hypothesis: $\dot{e}^t = \dot{\varepsilon}^t(F^{\dot{a}t}, \rho^t_0, \eta^t)$
- Collagen Stress: $P^c = \rho^c_0 \frac{\partial \varepsilon^c}{\partial F^{c}e} F^{g e} - T$
  - Hyperelastic Material
  - Continuum stored energy function based on the Worm-like chain model
- Fluid Stress: $P^f = \rho^f_0 \frac{\partial \varepsilon^f}{\partial F^{f}e} F^{g f} - T$
  - Ideal Fluid
  - $\rho^f_0 \dot{\varepsilon}^f = \frac{1}{2} \kappa (\text{det}(F^{e f}) - 1)^2$, $\kappa$ - fluid bulk modulus
- Fluid flux relative to collagen
  $M^f = D^f (\rho^f_0 F^T g + F^T \nabla \chi \cdot P^f - \nabla \chi (e^f - \theta \eta^f))$
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 $D_{ij}^f = 1 \times 10^{-8} \delta_{ij}$, Han et al. [2000]
- “Artificial” sources: $\Pi^f = -k^f (\rho_0^f - \rho_{0\text{ini}}^f)$, $\Pi^c = -\Pi^f$
- Entropy of mixing: $\eta_{\text{mix}}^f = -\frac{k}{M^f} \log \frac{\rho_0^f}{\rho_0}$
Example: Growth in a Bath

Stress (Pa) vs Extension (m)
Native Tendon is Functionally Graded

Two week old TA tendon
Tendon Growth is Not Homogeneous

How could this be modelled?
Choices for Volumetric Sources

- Simple first order rate law – Constituents either “solid” or “fluid”
  \[ \Pi^f = -k^f (\rho^f - \rho^f_{ini}), \quad \Pi^c = -\Pi^f \]

- Strain Energy Dependencies – Weighted by relative densities
  \[ \Pi^c = \left( \frac{\rho^c}{\rho^c_{ini}} \right)^{-m} \Psi_0 - \Psi_0^* \]
  [Harrigan & Hamilton, 1993]

- Enzyme Kinetics – Introducing additional species to the mixture
  \[ \Pi^s = \frac{(\Pi^{s}_{\text{max}} \rho^s)}{(\rho^s_{m} + \rho^s)} \rho_{cell}, \quad \Pi^c = -\Pi^s \]
  [Michaelis & Menten, 1913]

- Cell Signalling – Preferential growth in damaged regions
Viscoelastic Response of TA Tendon

Five continuous cycles, 0.01/s, 20 s delay
10 Minute recovery, Sixth cycle at 0.01/s
Regional VariationManifested in Viscoelastic Response of TA Tendon
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
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