Mechanical characterization of collagen fibers and scaffolds for tissue engineering

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Biomaterials

“All those materials used in medical devices in which contact with the tissues of the patient is an important and guiding feature of their use and performance.”

- Science and engineering aspects of biomaterials
  - mechanical, physical, chemical, biological properties

- Applications of biomaterials
  - implantable medical devices, tissue engineering and drug delivery systems
  - design, production, clinical performance characteristics
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  Ph.D Rensselaer Polytechnic Institute 1996  
  □ Cell and tissue engineering, biomaterials, cell adhesion, engineering education  
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  Ph.D University of Pittsburgh 1996  
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- **Eric A. Nauman**, Assistant Professor  
  Ph.D University of California at Berkeley 2000  
  □ Tissue engineering of bone and nerve tissue, degenerative diseases, mechanical loading of cells, mechanics of hierarchical materials, dynamics of biological systems
“To promote the progress of science; to advance the national health, prosperity, and welfare; and to secure the national defense.”

- Independent agency of U.S. Government
- National Science Board of 24 part-time members and Director appointed by the President with advice from the Senate
Background: Ligaments

- Connect bone to bone
- Provide stability
- Collagen fibers
- Heals poorly
- Autograft, allograft, TE
Background: Mechanical Properties

- Structural – specimen scale dependent
- Material – characteristic of material
  - tangent modulus: measure of stiffness taken from slope of linear region on stress-strain curve
- Viscoelastic – time dependent
  - stress-relaxation: “instantaneously” strain specimen, measure stress, which decreases with time
  - creep: apply and maintain constant stress (load), measure strain, which increases with time
Introduction

- Collagen gels
  - Cells produce ECM and aligned properly
  - Insufficient mechanical strength as tissue replacement

- Assessed structural, material, viscoelastic properties of single- and multi-fiber collagen scaffolds, addressing fiber diameter and source

- Studied effects of cells on mechanical properties of fiber-embedded gel scaffolds
Methods: General Outline

- Preparation of materials
  - Single collagen fibers
  - Fiber scaffolds
  - Fiber-embedded gel scaffolds

- Mechanical testing
  - Determination of fiber diameter
  - Tensile testing
  - Viscoelastic testing
Methods: Single Collagen Fibers

- Bovine achilles tendon Collagen Type I prepared and extruded through microbore tubing, diameters 0.051, 0.102, 0.127 cm
- Air dried overnight, reducing diameters by 1/10
- Fibers crosslinked by soaking in EDC
- Rat tail tendon collagen fibers used as a comparison material
**Methods: Fiber Scaffolds**

- Bovine/rat made in a similar way
- 10 fibers (7.6 cm long) aligned in parallel array and ends knotted
- Determined viability as cell culture substrates
- Seeded scaffolds with rat skin fibroblasts and cultured for 1, 2, 4, 8, 16 days
- Determined viability with “Live/Dead” stain at each time

*Not from this study just an illustration of “Live/Dead”*
Methods: Fiber-Embedded Gel Scaffolds

- Extruded collagen fibers combined with collagen gel
- 50 extruded collagen fibers (2.5 cm long) knotted
- Scaffolds placed into custom-built molds
- Fibroblast/collagen gel mixture poured into molds
- Incubated 30 min. then covered with cell culture medium and cultured
- Cell viability determined by “Live/Dead” assay
Mechanical Testing

- Determination of fiber diameter
  - Diameters of 17 random wet rat tail tendon collagen fibers measured using laser micrometer
  - Predicted fiber diameters confirmed by manual measurements using micrometer and light microscope
Mechanical Testing Cont.

- **Tensile testing**
  - Computer-controlled testing system (Instron Model 1122)
  - Tested at 12.7 cm/min loading rate
  - Some non-crosslinked scaffolds constructed of rat tail fibers loaded at rate of 2.54 cm/min
  - Samples kept hydrated by spraying with PBS during testing
  - Produce stress-strain curve and calculated tangent modulus
Mechanical Testing Cont.

- **Viscoelastic testing**
  - Tensile creep testing at 2.5 MPa
  - Samples kept hydrate by spraying with PBS
  - Measured elongation by LVDT (linear variable differential transformer)
  - Measured two parameters of creep
    - equilibration time
    - equilibrium strain
Results & Discussion

- **Determination of fiber diameter**
  - Rat tail tendon diameter: average value $271 \mu m$
  - Extruded collagen fiber diameter:
    - Tube diameter-510 $\mu m$, fiber (wet) diameter $59 \mu m$
    - Tube diameter-1020 $\mu m$, fiber (wet) diameter $125 \mu m$
    - Tube diameter-1270 $\mu m$, fiber (wet) diameter $158 \mu m$
  - Used the following equation:
    $\text{Wet Fiber } \varnothing (\mu m) = \{0.1298 \times \text{Extrusion tube } \varnothing (\mu m)\} - 6.79 \mu m$
Results & Discussion Cont.

- **Tensile Testing**
  - Modulus and peak stress decreased as the diameter of extruded crosslinked fibers increased
  - Crosslinked rat tail tendon had a much larger modulus and peak stress compared to the extruded fibers

- Larger fibers are more likely to include defects and has a smaller surface to volume ratio

<table>
<thead>
<tr>
<th>Source</th>
<th>Diameter (μm)</th>
<th>n</th>
<th>Modulus (MPa)</th>
<th>Peak stress (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extruded</td>
<td>59</td>
<td>8</td>
<td>484.7 ± 76.3</td>
<td>50.0 ± 13.4</td>
</tr>
<tr>
<td>Extruded</td>
<td>125</td>
<td>11</td>
<td>359.6 ± 28.4</td>
<td>36.0 ± 5.4</td>
</tr>
<tr>
<td>Extruded</td>
<td>158</td>
<td>10</td>
<td>269.7 ± 11.9</td>
<td>24.7 ± 2.9</td>
</tr>
<tr>
<td>Rat tail tendon</td>
<td>271</td>
<td>12</td>
<td>1174.9 ± 283.3</td>
<td>114.6 ± 51.0</td>
</tr>
</tbody>
</table>
Results & Discussion Cont.

- **Tensile Testing**
  - Top graph: stress-strain curve for a crosslinked, single, extruded fiber
  - Lower graph: stress-strain curve for a crosslinked, single, rat tail collagen fiber

- Although different shapes, both produce a classic stress-strain response characteristic of soft biological materials

- Rat tail exhibits strain softening
Tangent modulus of scaffolds from 14 non-crosslinked rat tail tendon fibers depended on initial length of scaffold and rate of load application.

Significant observation since tangent moduli is a material property that should not depend on overall specimen size yet this graph shows that it is dependent.

Behavior also observed for many viscoelastic soft tissues.

- **Extension rate of 2.54 cm/min**
  - \[ y = 23.177x + 2.6449 \]
  - \[ R^2 = 0.6272 \]

- **Extension rate of 12.7 cm/min**
  - \[ y = 36.137x - 14.743 \]
  - \[ R^2 = 0.8905 \]
Results & Discussion Cont.

Table 2
Mechanical properties of collagen scaffolds as a function of fiber number

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of fibers</th>
<th>$n$</th>
<th>Modulus (MPa)</th>
<th>Peak stress (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extruded</td>
<td>1</td>
<td>11</td>
<td>359.6 ± 28.4</td>
<td>36.0 ± 5.4</td>
</tr>
<tr>
<td>Extruded</td>
<td>10</td>
<td>12</td>
<td>261.2 ± 63.5</td>
<td>19.9 ± 7.2</td>
</tr>
<tr>
<td>Rat tail tendon</td>
<td>1</td>
<td>12</td>
<td>1174.9 ± 283.3</td>
<td>114.6 ± 51.0</td>
</tr>
<tr>
<td>Rat tail tendon</td>
<td>10</td>
<td>13</td>
<td>995.1 ± 144.0</td>
<td>106.1 ± 13.9</td>
</tr>
</tbody>
</table>

- **Tensile Testing:**
  - Scaffolds with 10 fibers of extruded collagen with diameters 125 µm had a modulus and peak stress significantly less than those of 1 fiber
  - No significant variation between scaffolds of 10 fibers and 1 fiber for rat tail tendon collagen
- **Discussion Question:** What do you believe is the significance and meaning of the data in this table?
Results & Discussion Cont.

**Table 3**  
Mechanical properties of collagen scaffolds cultured with and without cells

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Modulus (MPa)</th>
<th>Peak stress (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without cells</td>
<td>5</td>
<td>49.6 ± 3.3</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>With cells</td>
<td>6</td>
<td>83.4 ± 10.8*</td>
<td>5.4 ± 0.4*</td>
</tr>
</tbody>
</table>

- **Tensile Testing:**
  - After 25 days of culture, fiber-embedded gels (125 µm Ø) containing cells exhibited significantly higher tangent moduli and peak stress values when compared to gels without cells

- **Discussion Question:** What are some possible reasons for the altered mechanical properties of cell seeded scaffolds?
Results & Discussion Cont.

- (A) stress-strain curve for fiber-embedded gels without cells
- (B) stress-strain curve for fiber-embedded gels with cells
- Note how (B) is more uniform and contains fewer incremental failures indicated by arrows in (A)
Results & Discussion Cont.

- **Creep Test:**
  - Mean equilibrium time for creep-tested 10-fiber extruded collagen scaffolds was $30.02\pm1.33s$
  - Mean equilibrium strain was $0.095\pm0.024$

- Viscoelastic creep here is very rapid compared to actual ligaments where creep continues beyond 20 min.

- Suggests that viscoelastic behavior of soft tissues is controlled by more than just collagen

- Scaffolds in this study were made of Collagen Type I while native ligaments are composed also of other ECM components
Discussion

- Rat tail tendon is biologically derived and well studied as a source of collagenous tissue, which is often used as a control or reference biomaterial.

- Intend to create scaffolds for replacing normal human ligament tissue.

- Lack of literature on mechanical properties of human knee ligaments.
Some studies report the ACL/PCL having a modulus of 345 MPa and peak stress of 36.4 MPa.

125 µm diameter single fibers exhibited similar properties, but scaffolds of these fibers (multi-fiber) showed decreased material properties (modulus 261.2 MPa; peak stress 19.9MPa)

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Conclusion

- Peak stress should not be the main material property considered in ligament design, but should be considered as a factor of safety.

- One should work to match the properties of the engineered tissue to the natural tissue in the low-end of the stress strain curve. This is the area where most physiological loading occurs.
Conclusion Cont.

- To develop novel collagen gel/scaffold constructs, one must have an understanding of the mechanical properties of the components.

- The data presented in this study was a stepping stone in understanding the mechanical properties of single fibers and collagen scaffolds.

- Future work is necessary to understand the contribution of cells and to understand the effect of gauge length on the modulus.
Fun Stuff

- Fossilized Tyrannosaurus Rex Collagen!