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

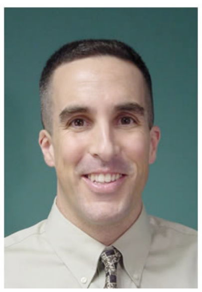
Authors (Tulane University Dept. of Biomedical Engineering)

- **Kay C. Dee**, Associate Professor

  Ph.D Rensselaer Polytechnic Institute 1996

  - Cell and tissue engineering, biomaterials, cell adhesion, engineering education

  Eileen Gentleman and Andrea N. Lay



- **Glen A. Livesay**, Assistant Professor

  Ph.D University of Pittsburgh 1996

  - Experimental and theoretical mechanics, soft tissue and joint mechanics, engineering education

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- **Eric A. Nauman**, Assistant Professor

  Ph.D University of California at Berkeley 2000

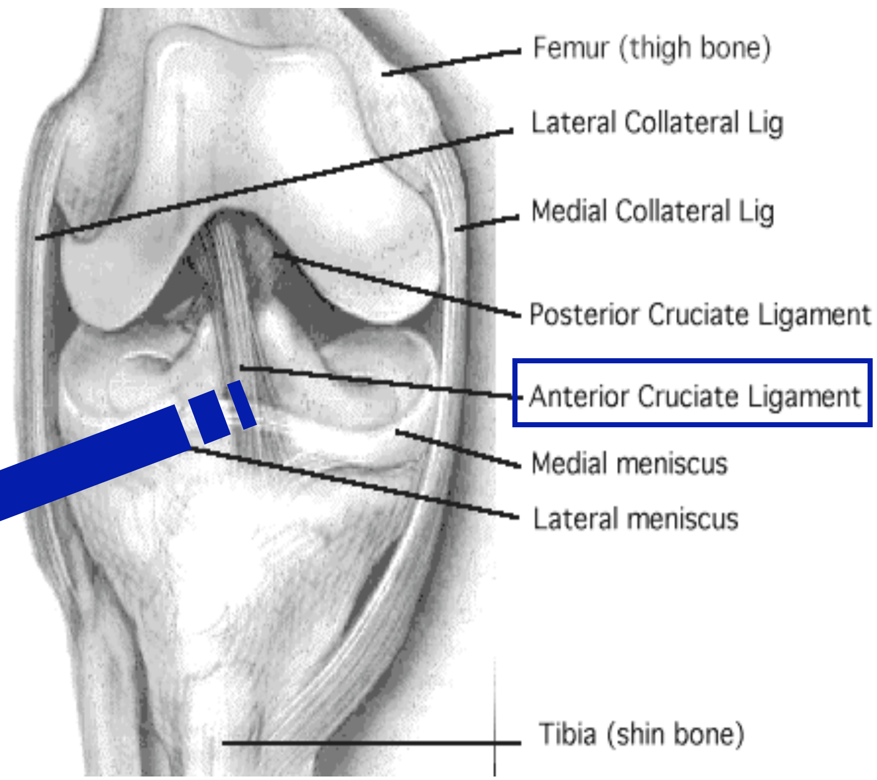
  - Tissue engineering of bone and nerve tissue, degenerative diseases, mechanical loading of cells, mechanics of hierarchical materials, dynamics of biological systems

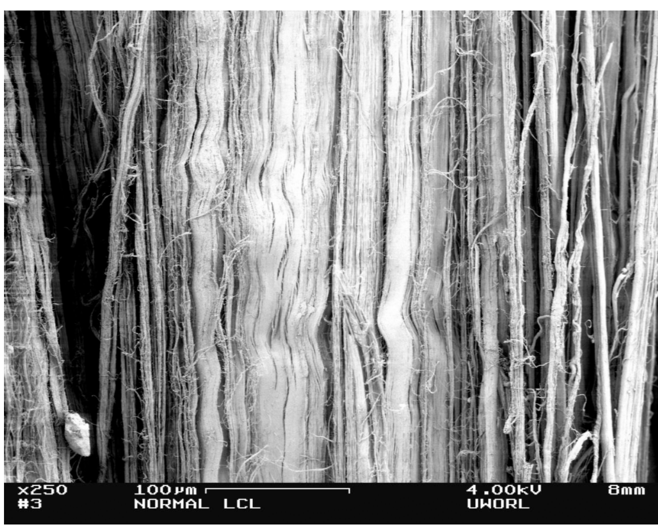
"To promote the progress of science; to advance the national health, prosperity, and welfare; and to secure the national defense."

- 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

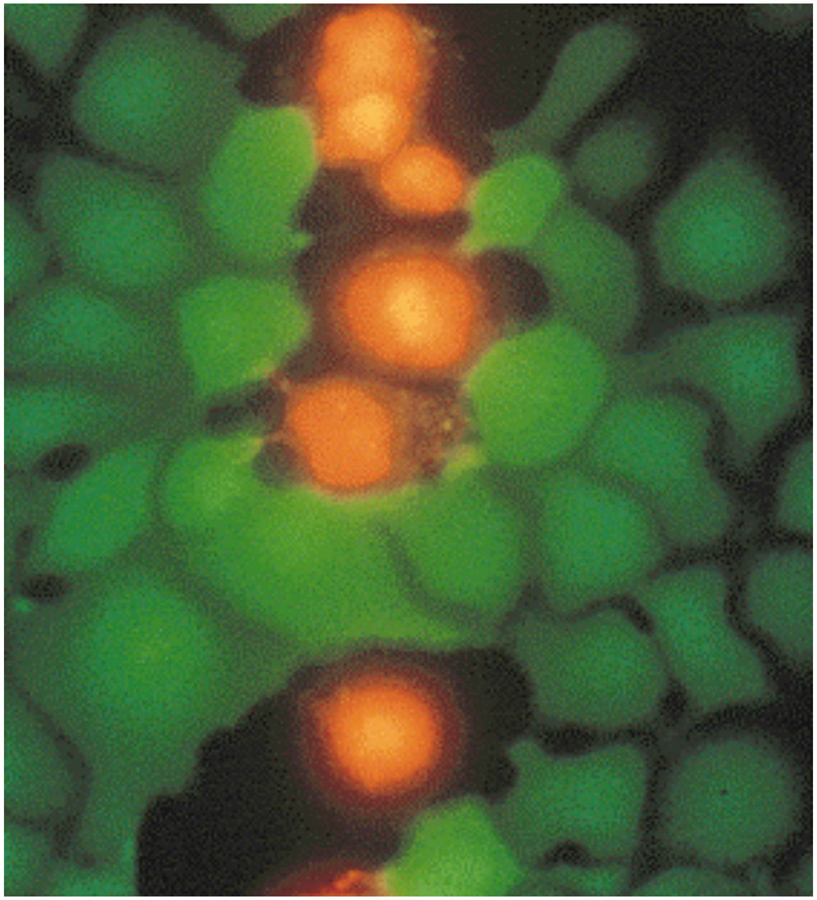
- Preparation of materials
  - Single collagen fibers
  - Fiber scaffolds
  - Fiber-embedded gel scaffolds
- Mechanical testing
  - Determination of fiber diameter
  - Tensile testing
  - Viscoelastic testing

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



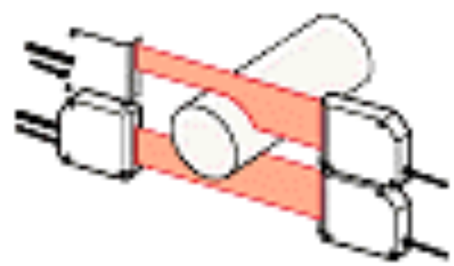
* Not from this study just an illustration of "Live/Dead"

Methods: Fiber-Embedded Gel Scaffolds

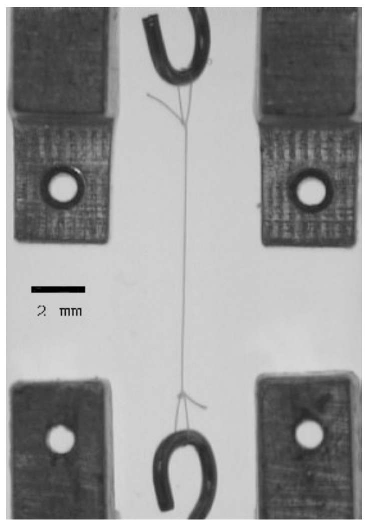
- Extruded collagen fibers combined with collagen gel
- 50 extruded collagen fibers (2.5 cm long) knotted
- Scaffolds placed into custom-built molds
- Fibroblast/collagen gel mixture poured into molds
- Incubated 30 min. then covered with cell culture medium and cultured
- Cell viability determined by “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 271μm
  - Extruded collagen fiber diameter:
    - Tube diameter-510 μm, fiber (wet) diameter 59 μm
    - Tube diameter-1020 μm, fiber (wet) diameter 125 μm
    - Tube diameter-1270 μm, fiber (wet) diameter 158 μm
    - Used the following equation:

      Wet Fiber Ø (μm) = {0.1298 x Extrusion tube Ø (μm)} - 6.79 μm

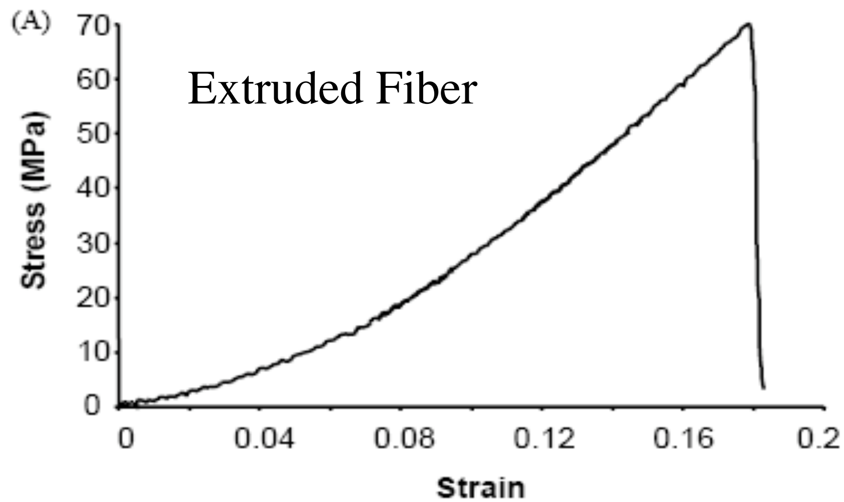
Results & Discussion Cont.

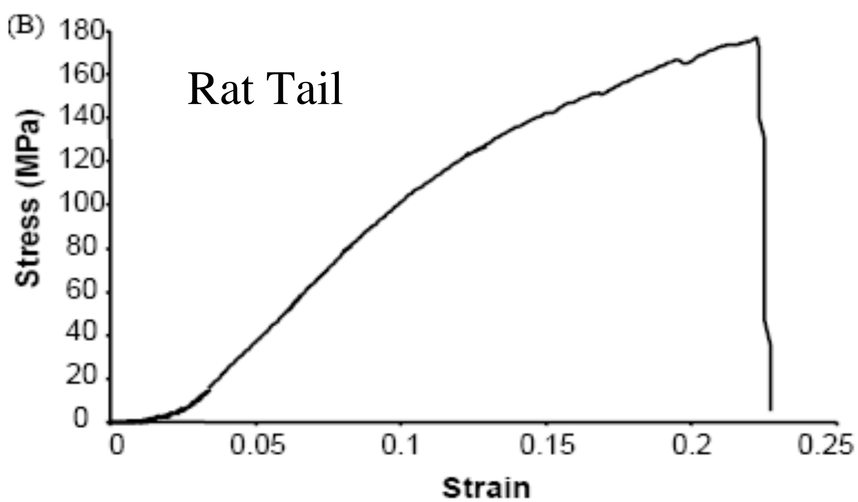
Table 1
Mechanical properties of crosslinked, single collagen fibers

Source	Diameter (μm)	n	Modulus (MPa)	Peak stress (MPa)
Extruded	59	8	484.7 ± 76.3	50.0 ± 13.4
Extruded	125	11	359.6 ± 28.4	36.0 ± 5.4
Extruded	158	10	269.7 ± 11.9	24.7 ± 2.9
Rat tail tendon	271	12	1174.9 ± 283.3	114.6 ± 51.0

- 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

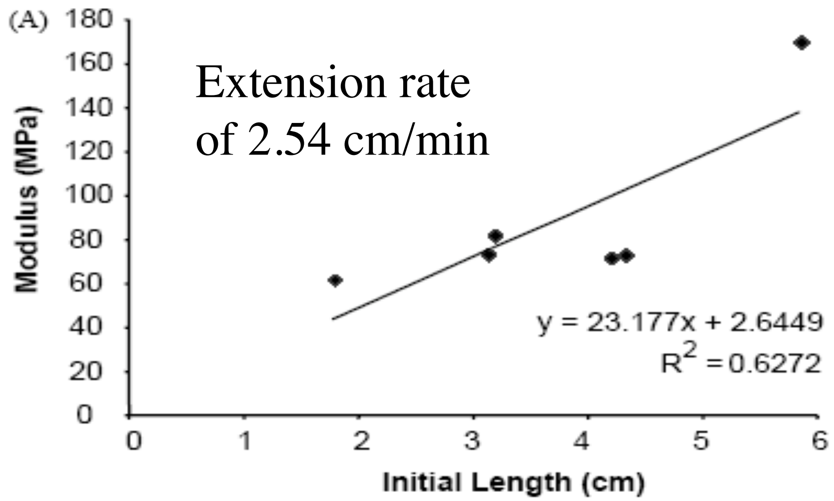
Results & Discussion Cont.

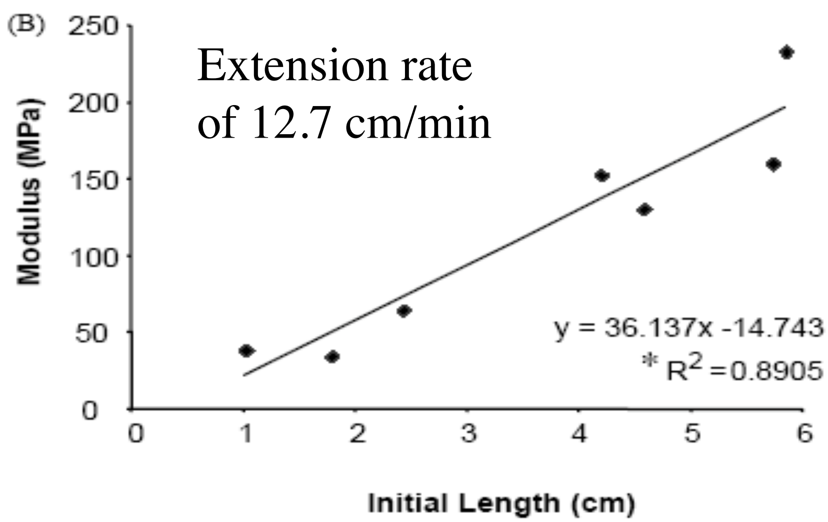




- Tensile Testing
  - Top graph: stress-strain curve for a crosslinked, single, extruded fiber
  - Lower graph: stress-strain curve for a crosslinked, single, rat tail collagen fiber
- Although different shapes, both produce a classic stress-strain response characteristic of soft biological materials
- Rat tail exhibits strain softening

Results & Discussion Cont.





- 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 <u>should not</u> depend on overall specimen size yet this graph shows that it <u>is dependent</u>
- Behavior also observed for many viscoelastic soft tissues

Results & Discussion Cont.

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

Source	Number of fibers	n	Modulus (MPa)	Peak stress (MPa)
Extruded	1	11	359.6 ± 28.4	36.0 ± 5.4
Extruded	10	12	261.2 ± 63.5	19.9 ± 7.2
Rat tail tendon	1	12	1174.9 ± 283.3	114.6 ± 51.0
Rat tail tendon	10	13	995.1 ± 144.0	106.1 ± 13.9

- 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?

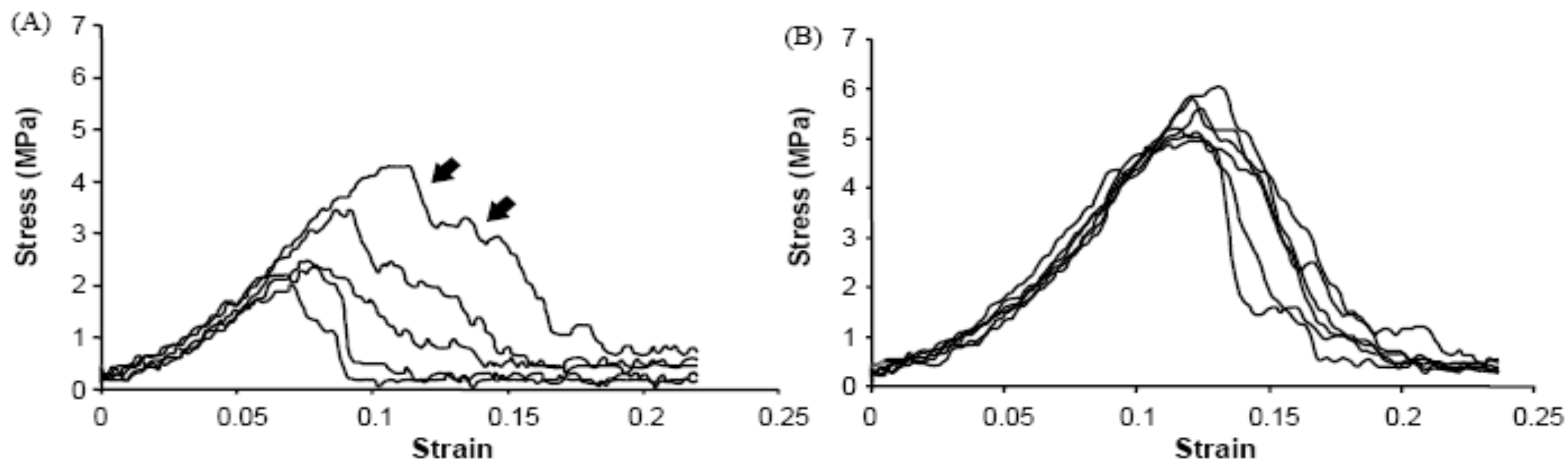
Results & Discussion Cont.

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

	N	Modulus (MPa)	Peak stress (MPa)
Without cells	5	49.6 ± 3.3	2.9 ± 0.9
With cells	6	83.4 ± 10.8*	5.4 ± 0.4*

- 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±1.33s
  - Mean equilibrium strain was 0.095±0.024
- 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

Discussion Cont.

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Mechanical properties of crosslinked, single collagen fibers

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- 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)

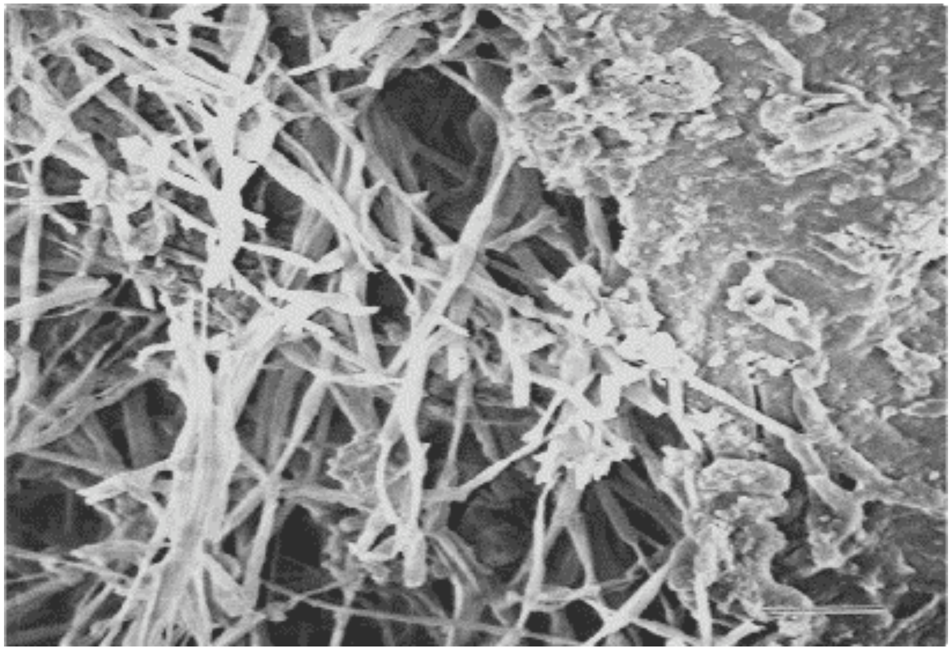
Conclusion

- 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!