

Silk, Scaffolds, and Stem Cells

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2008 Summer Experience

Virginia Commonwealth University BBSI

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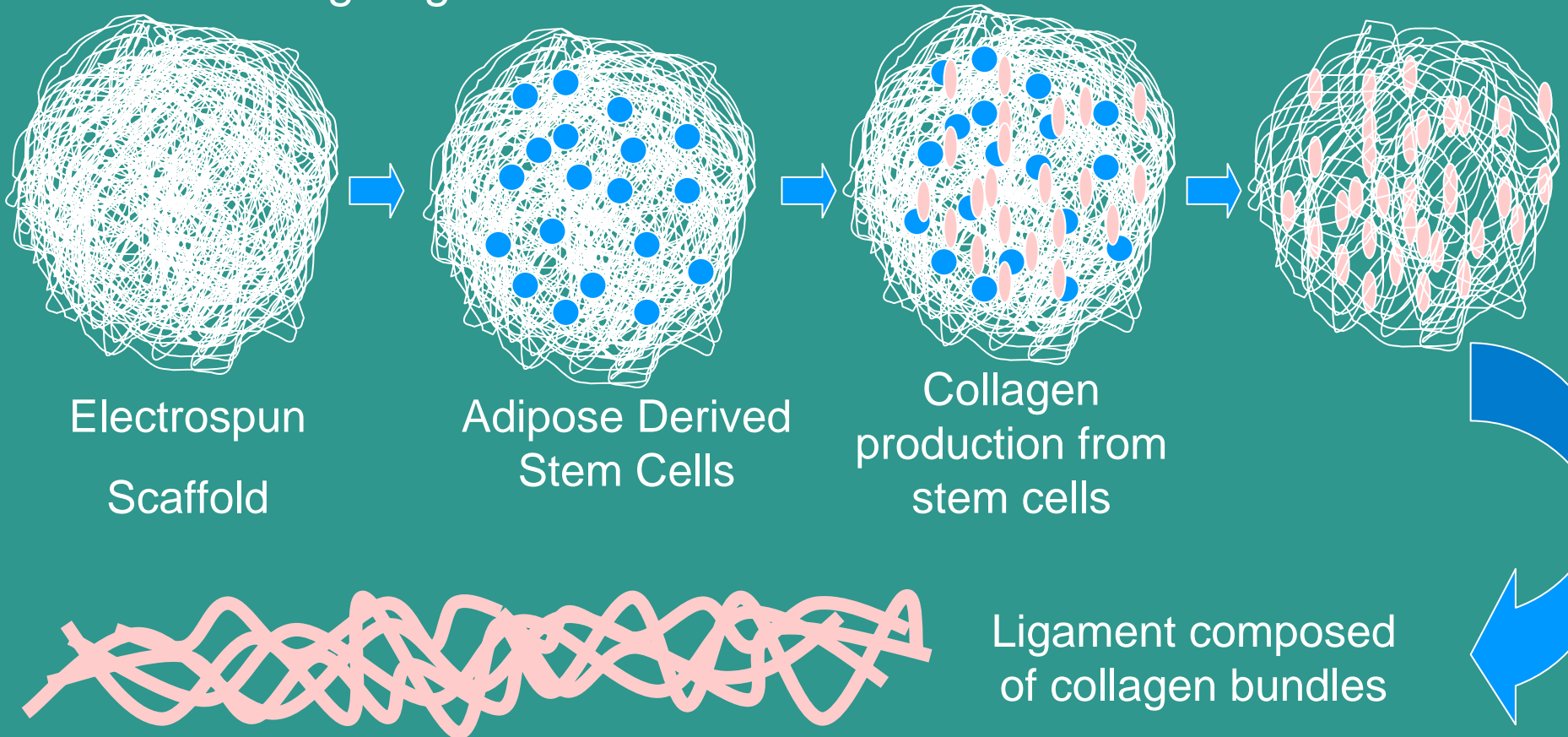
Need for Ligament Tissue Engineering

- Knee ligaments cannot self repair
- High injury rate, especially the anterior cruciate ligament (ACL)
 - > 200,000 ACL surgeries/year
 - > 5 billion dollars
- Surgery options
 - Disease transfer
 - Tissue rejection
 - Poor mechanical strength (current synthetic grafts)



Research on the Rise

- Design a scaffold that will mimic the native ACL tissue
 - Mechanical support
 - Collagen growth

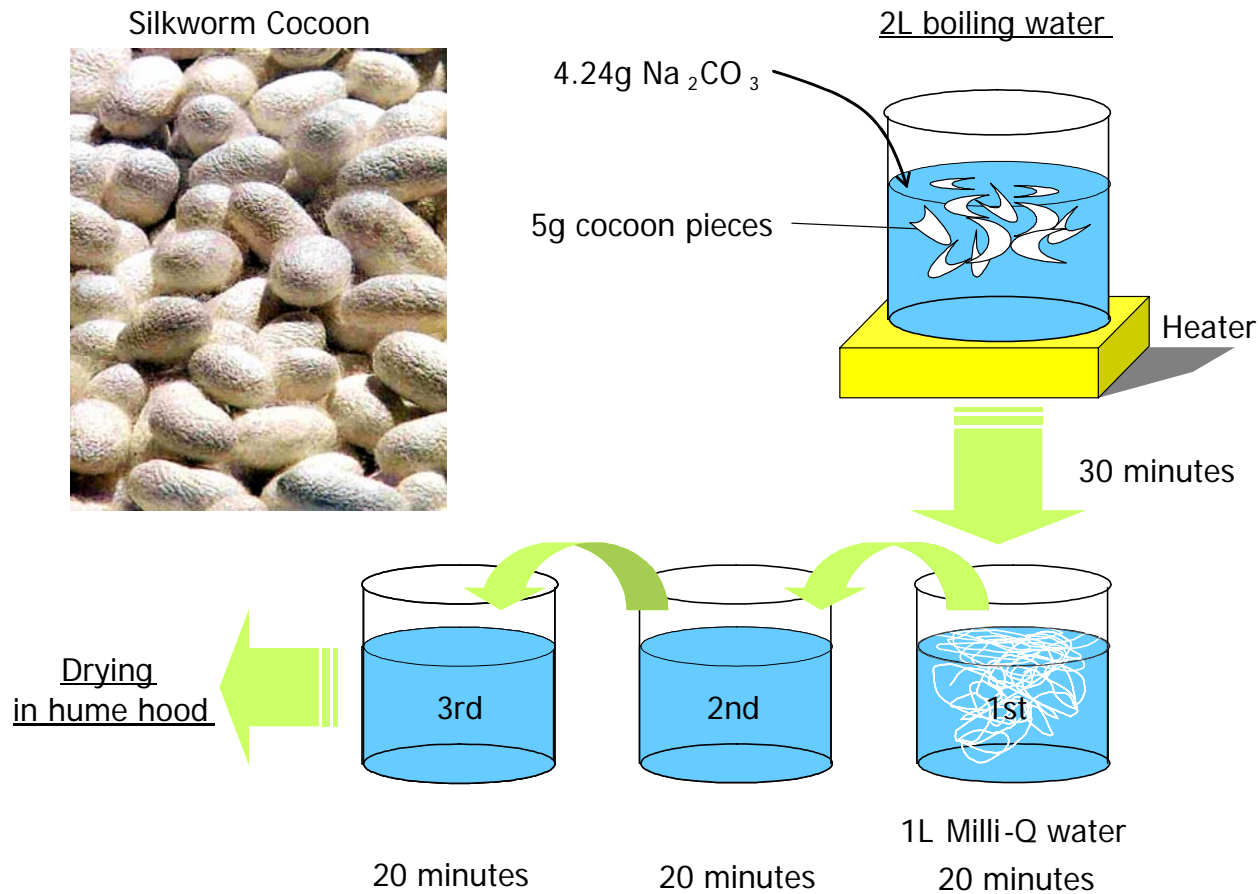


Silk Properties Yield a Promising Scaffold Choice

- Biocompatibility
- Fibroin protein polymer already spun into fibers by the Bombyx Mori silkworm
- Distinguishing mechanical properties
- Slow degradation in vivo

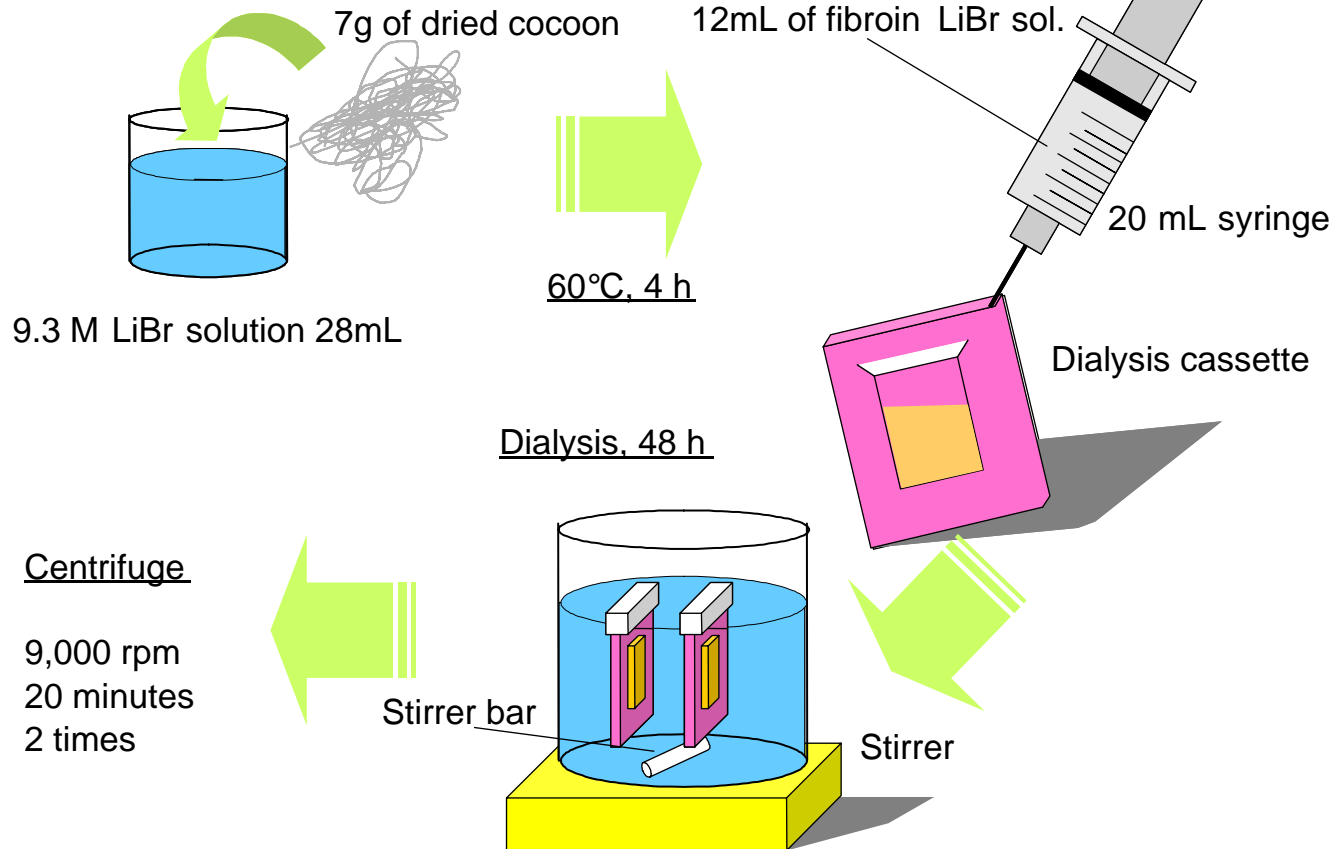


Silk Preparation from Cocoons



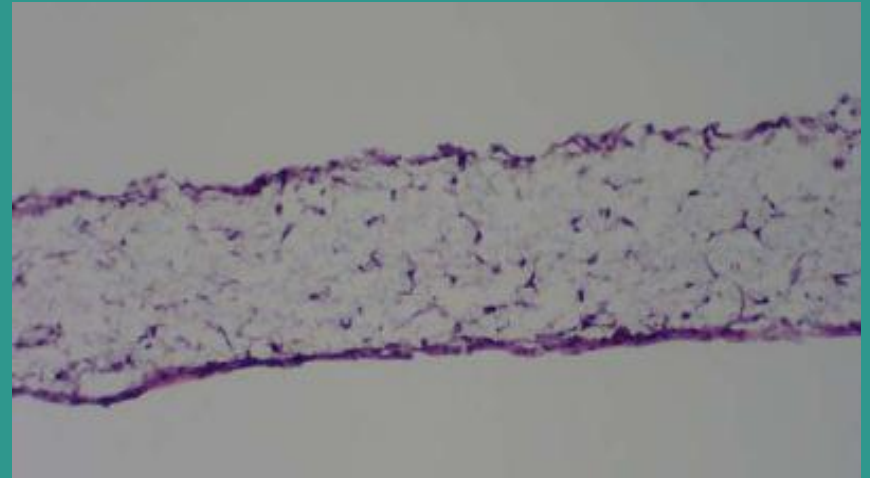
Removing Sericin from Silk

Dissolving in LiBr solution



PCL (Polycaprolactone) Properties Yield a Promising Scaffold Choice

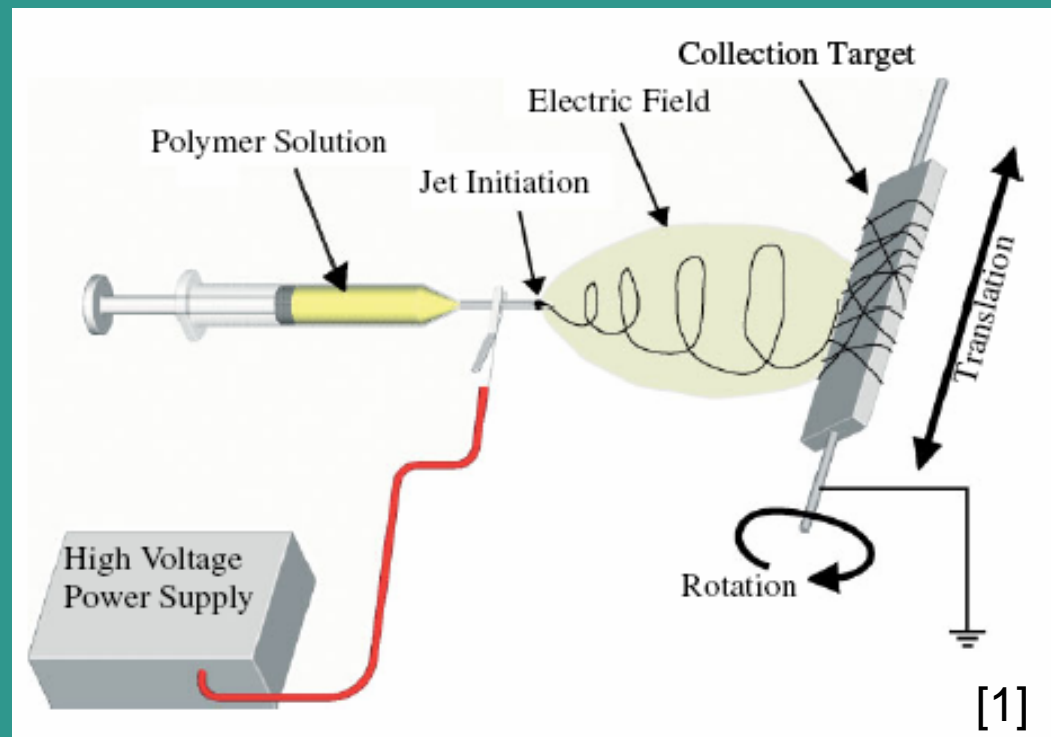
- Biocompatible
- Slow degradation in vivo
- Stretchy/mechanically strong polymer
- Able to formulate polymer blends



<http://web.mit.edu/rutledgegroup/projects/electrospinning.html>

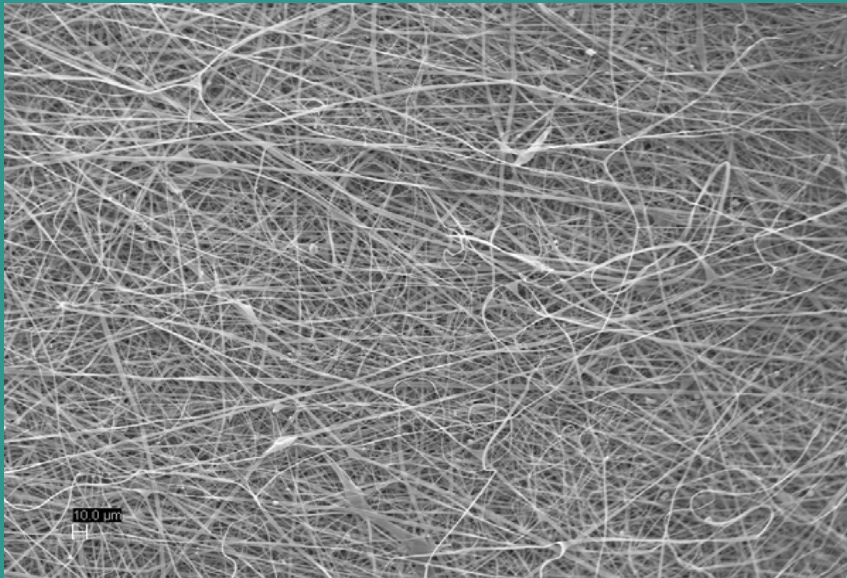
Electrospinning Polymers

- Electrospinning is just one novel of creating nanofiber polymer scaffolds that mimic the native tissues of the extracellular matrix [8]
- It yields a high surface area to volume ratio preferable for cellular infiltration

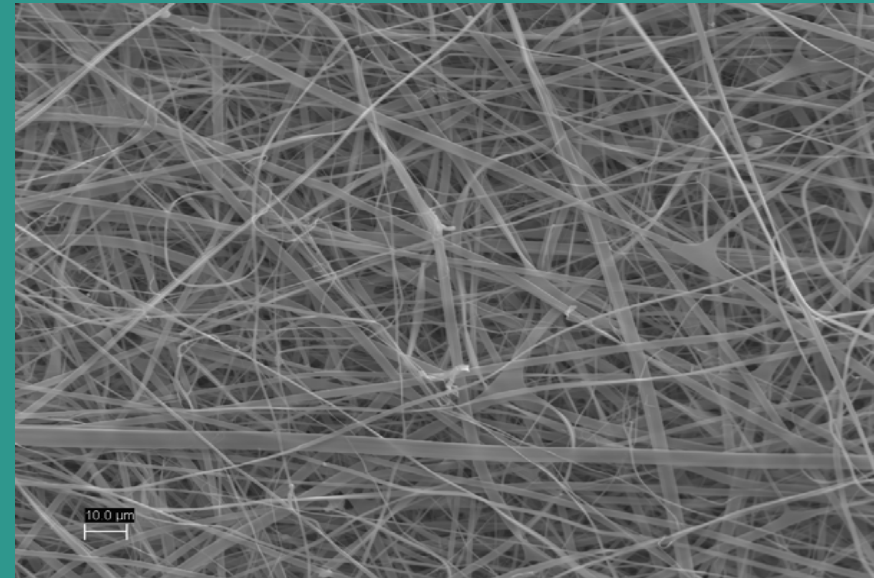


Electrospun Silk Scaffolds

Magnified 500X



Magnified 1500X



SEM Images

Punching the Silk and Silk/PCL Scaffolds

- 3 pure silk sheets were electrospun
- 2 50/50 (by volume) silk/PCL sheets were electrospun



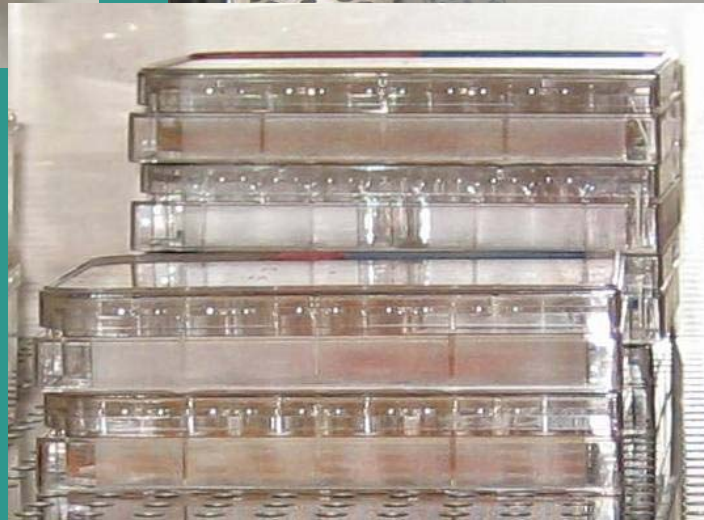
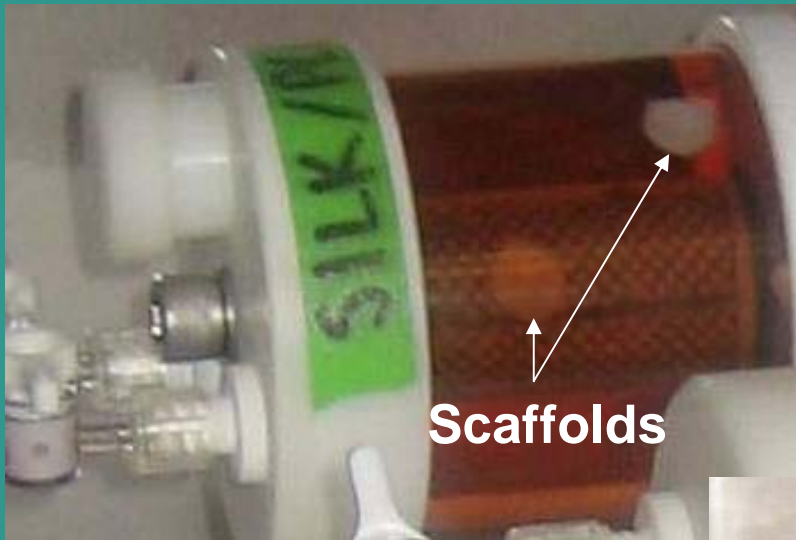
Do Not Let the Cells Die!

ADSCs (Adipose Derived Stem Cells)



Cells were fed culture media every 2 days and split into 3 separate flasks as each flask became confluent with cells

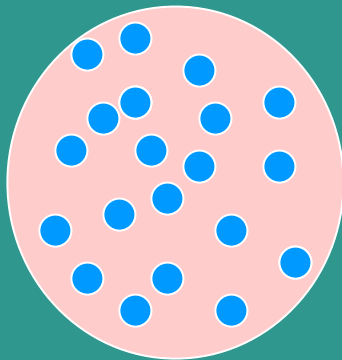
Culturing Stem Cells



Collagen Assay Testing on Disk Scaffolds at Day 7

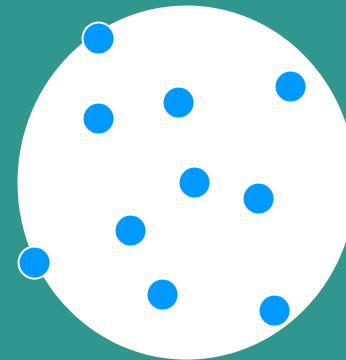
- If cells like their environment, they will produce collagen
- Before producing collagen, cells proliferate and migrate on the scaffolds

Cells are set on the scaffold



Collagen production

Cells are not yet situated

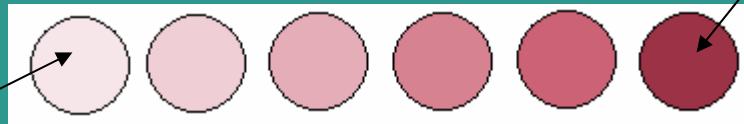


No collagen production

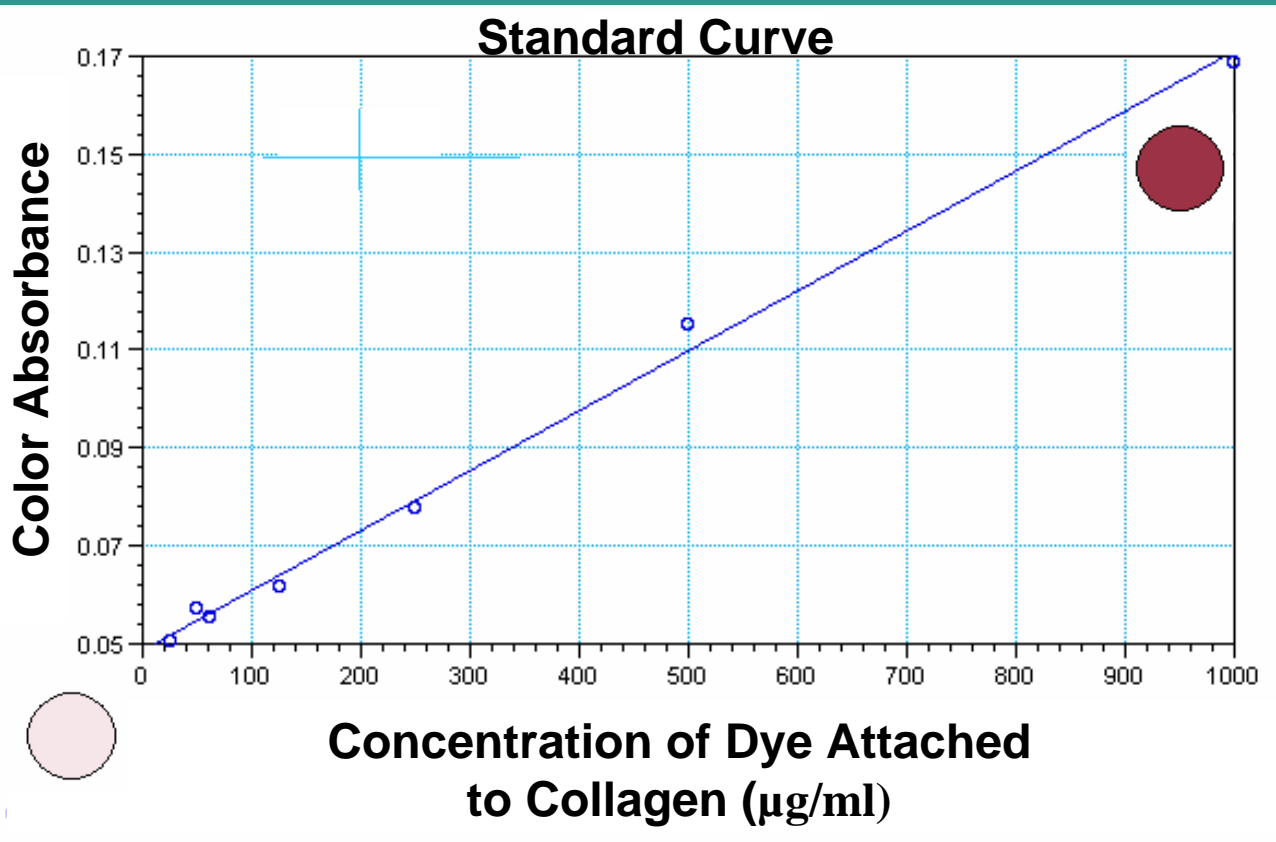
Hypothesis

Collagen Assay Standards are in Acceptable Range

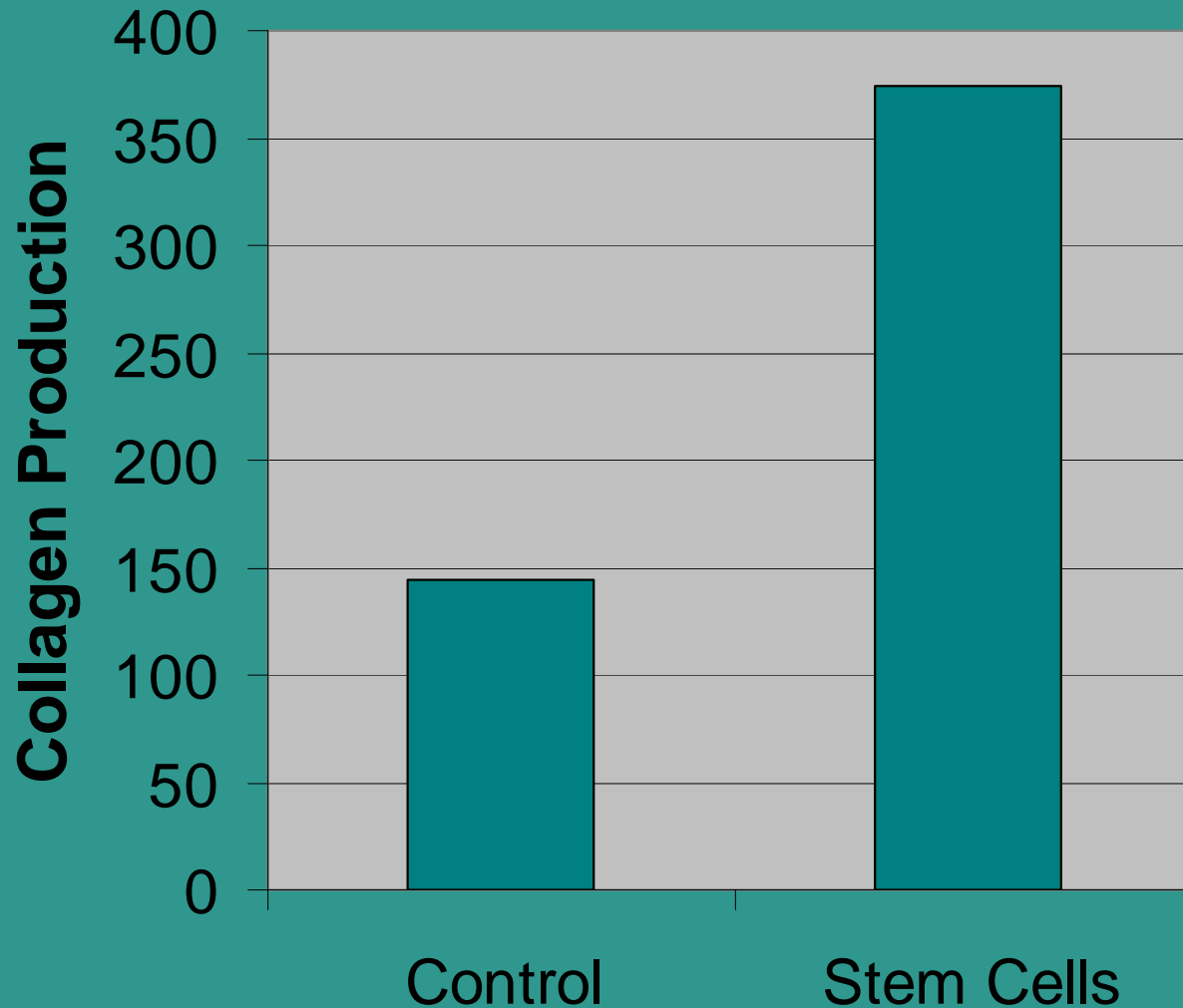
Minimal collagen detected on scaffold



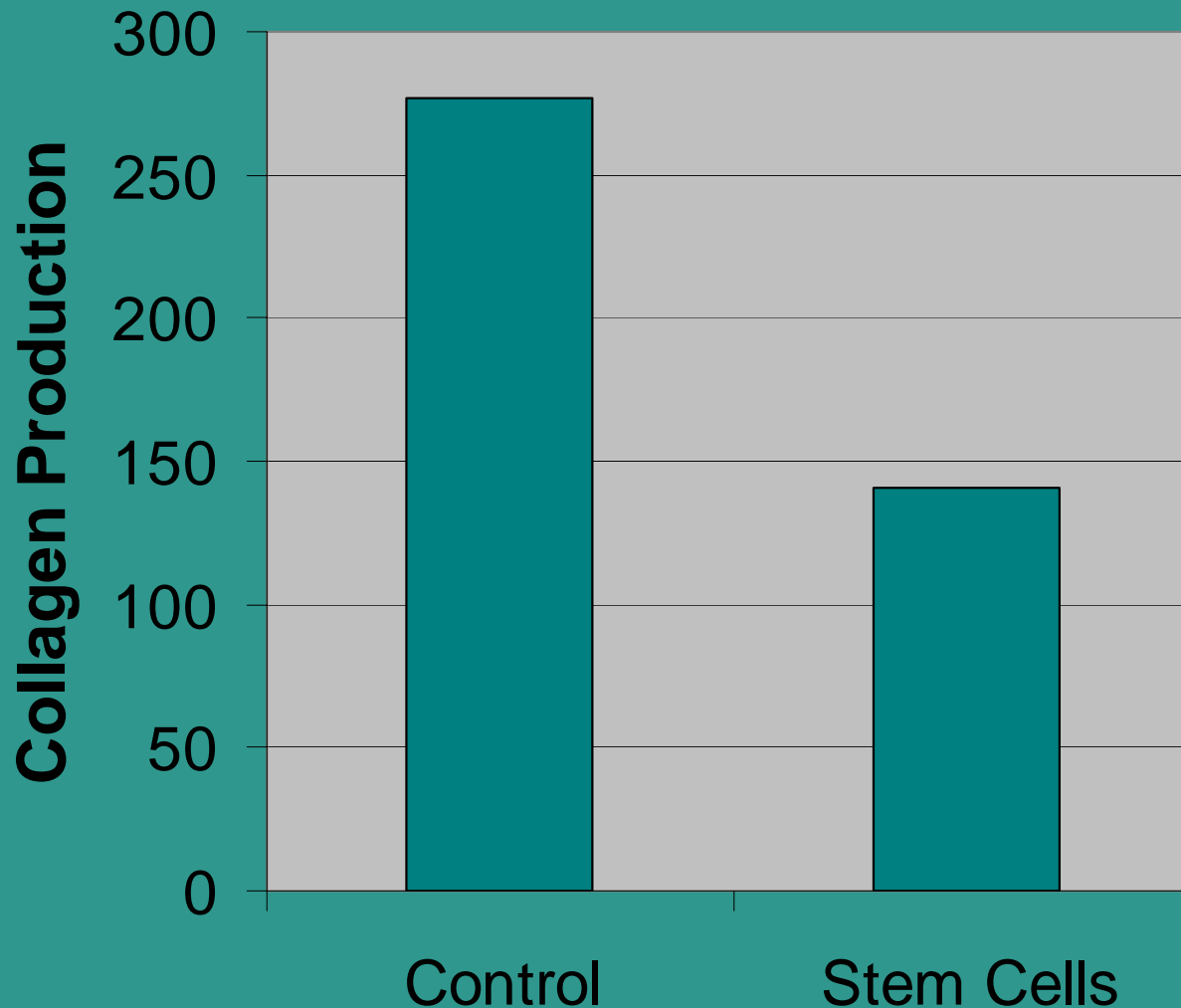
Lots of collagen detected on scaffold



Silk Collagen Assay Results After 1 Week



Silk/PCL Collagen Assay Results After 1 Week



Stretching the Scaffolds to Failure



Mechanical Properties of Ligaments

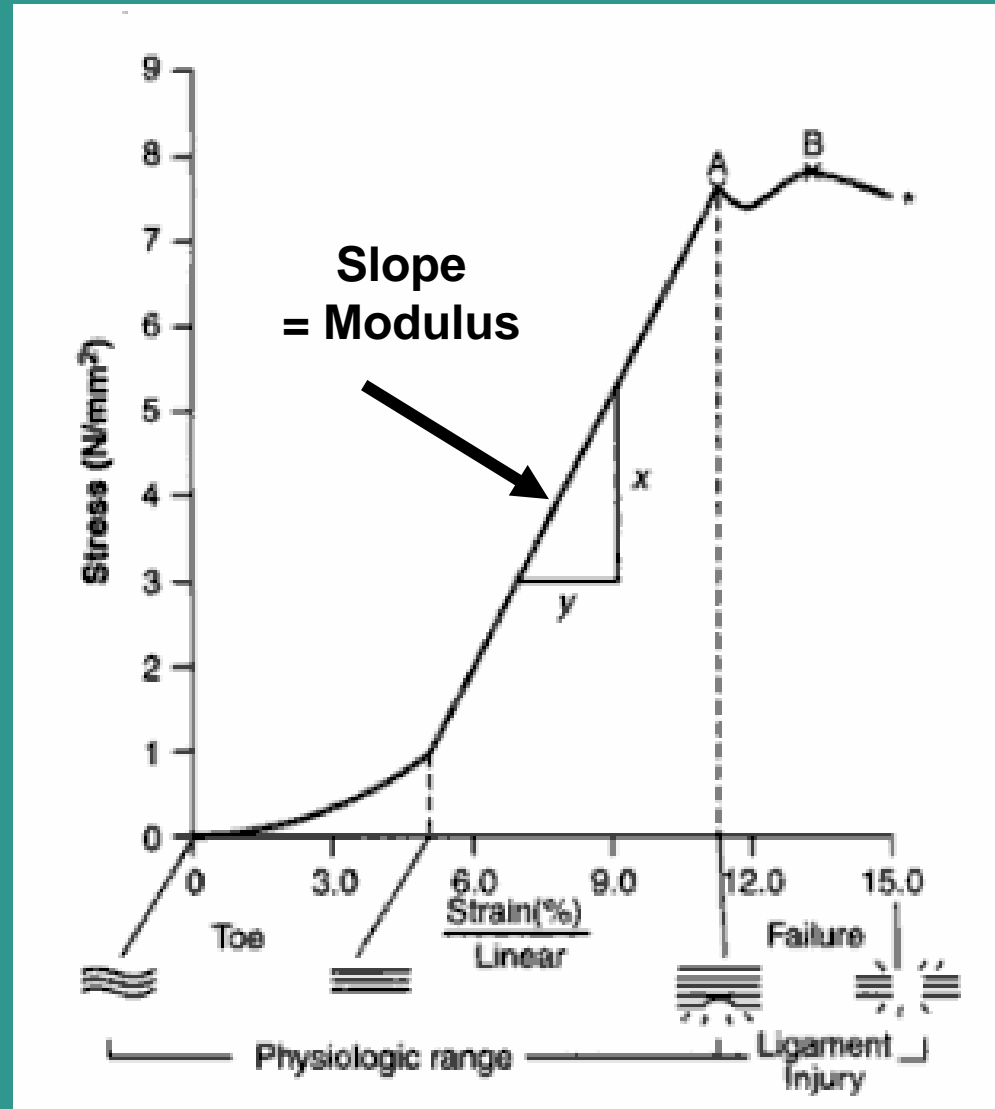
Elastic Regions

Toe Region: initial loading, collagen crimp is initially straightened in ligaments

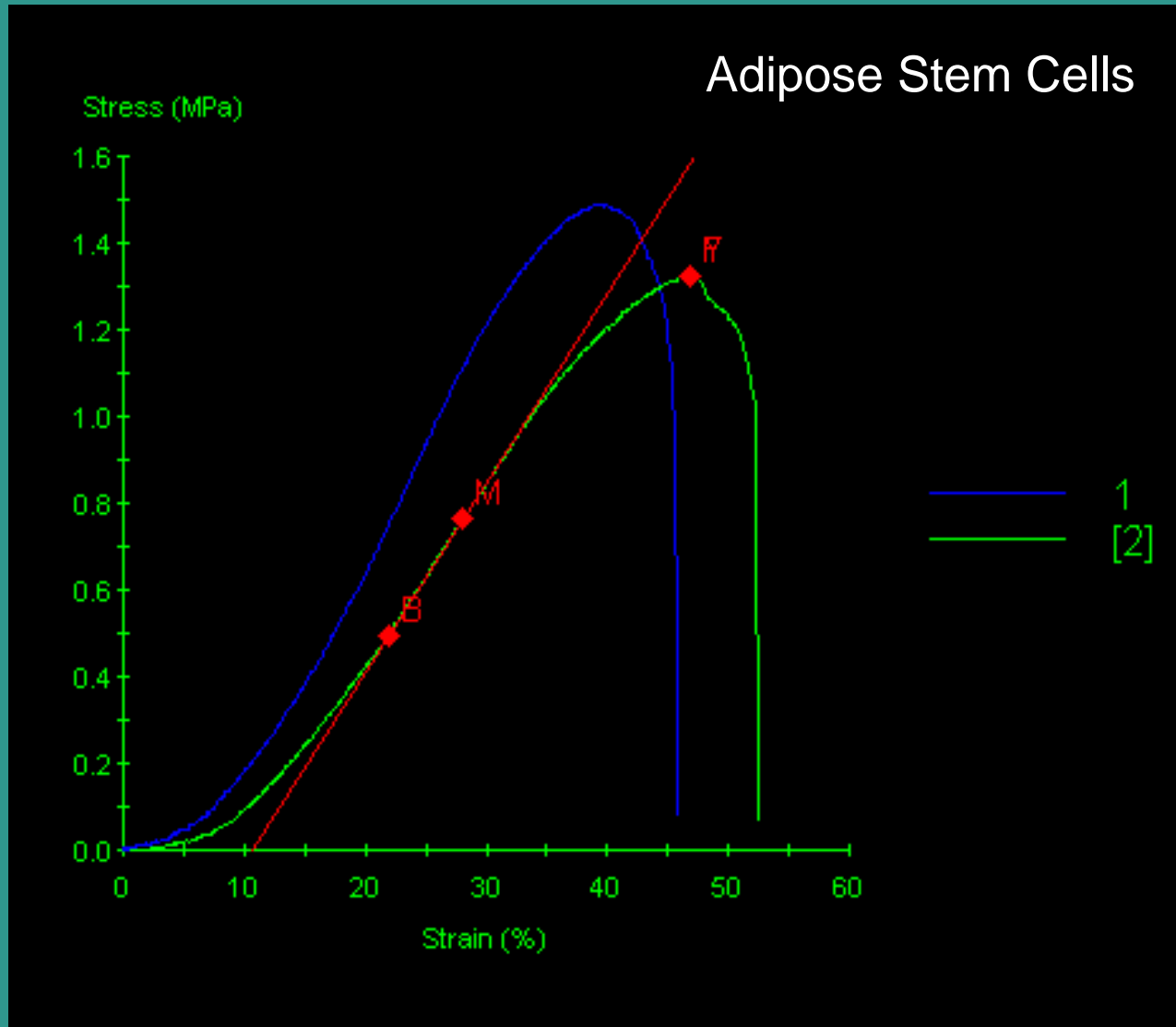
Linear Region: collagen crimp is completely straightened out

Plastic Region

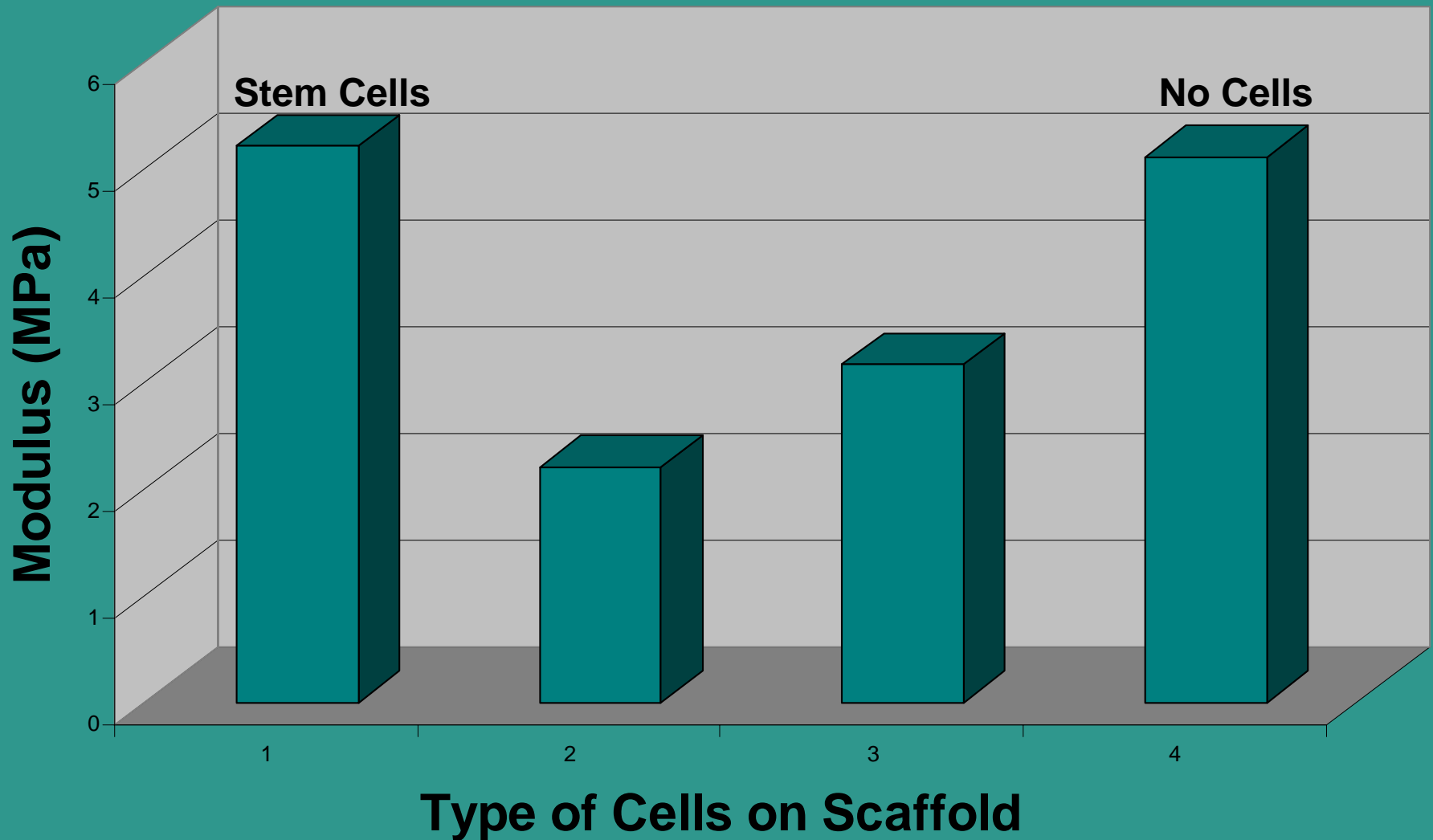
Ligament failure occurs when the collagen fibers break



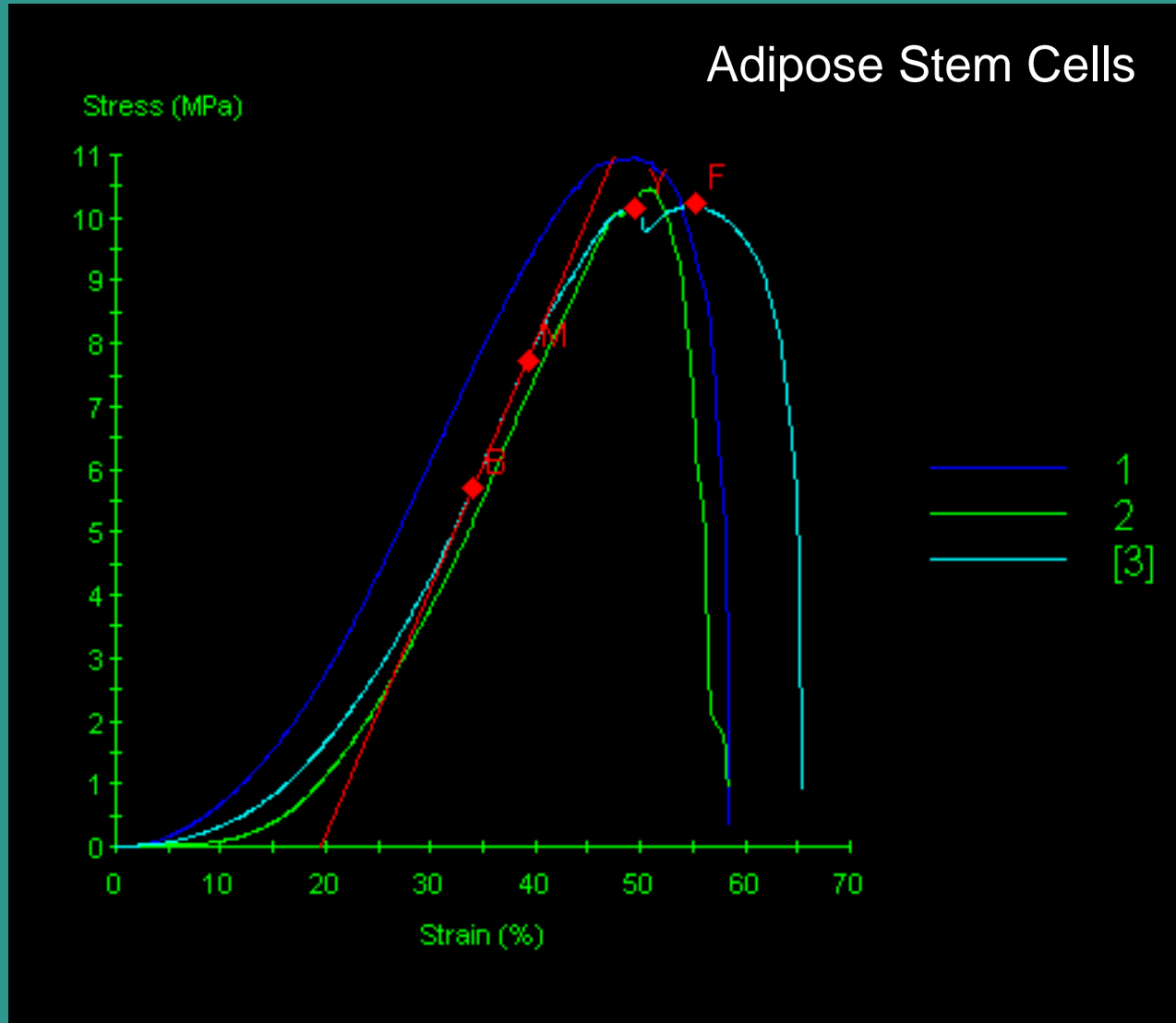
Silk Graphs Similar to Actual Ligament



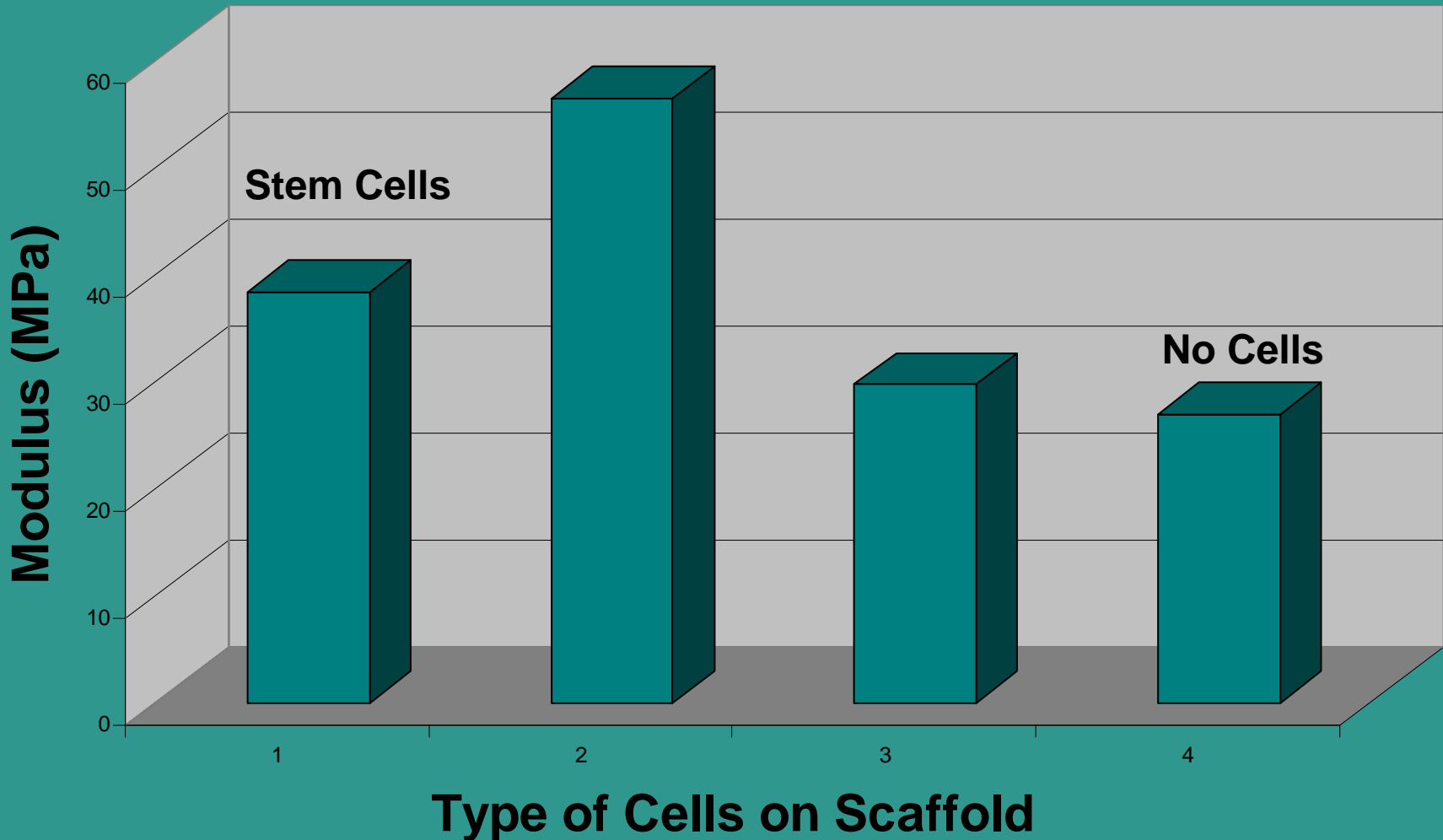
Mean Modulus for Silk/PCL Scaffolds 1 Week After Seeding Cells



Silk/PCL Graphs Resemble Ligament



Mean Modulus for Silk/PCL Scaffolds 1 Week After Seeding Cells



The Project is Still Underway...

- Histology pictures that show the ADSCs on the scaffolds should be in the mail very soon
- Day 14 results will be assembled today
- Day 21 results will be assembled next Tuesday

References

- [1] Sell S, Bowlin G, et al, *Polymer International* 56:1350,1358 (2007).
- [2] Bashur C, Dahlgren LA and Goldstein AS, *Biomaterials* 27:5681 (2006).
- [3] Lee CH, et al, *Biomaterials* 26:1261 (2005).
- [4] Altman, G H, Kaplan, DL, et al, *Biomaterials* 24:402-406 (2002).
- [5] Zhidao X, Triffitt JT, *Biomed. Matter* 1:R1-R9 (2006).
- [6] Zigang G, Lee EH, et al, *Journal of Biomedical Materials Research Part A* 77A: 639-652 (2006).
- [7] Functional Soft-Tissue Examination and Treatment by Manual Methods Author: Warren I. Hammer pp 18-20
- [8] *Bowlin G, Materials Today* 7:64 (2004).

Thanks for a BBSI Summer!

- Dr. Gary Bowlin for his lab, time, resources, and lunch!
- Graduate students for their time, patience, and helpful responses to my questions
 - Scott Sell, Koyal Garg, Tricia Wolfe, Michael McClure, Yas Maghdouri Moghaddam, Anna Bulysheva, Parth Madurantakam, and Michael Frances for the stem cells
- Dr. Jeff Elhai, Dr. Sherry Baldwin, and Billy Budd for directing VCU BBSI
- Dr. Wan-Ling Chiu for the Bombyx Mori silkworm cocoons
- National Science Foundation for the grant money!