Developing an Orientation-graded Nanomatrix to Promote Axonal Regeneration and Functional Recovery in Spinal Cord Injury (SCI)

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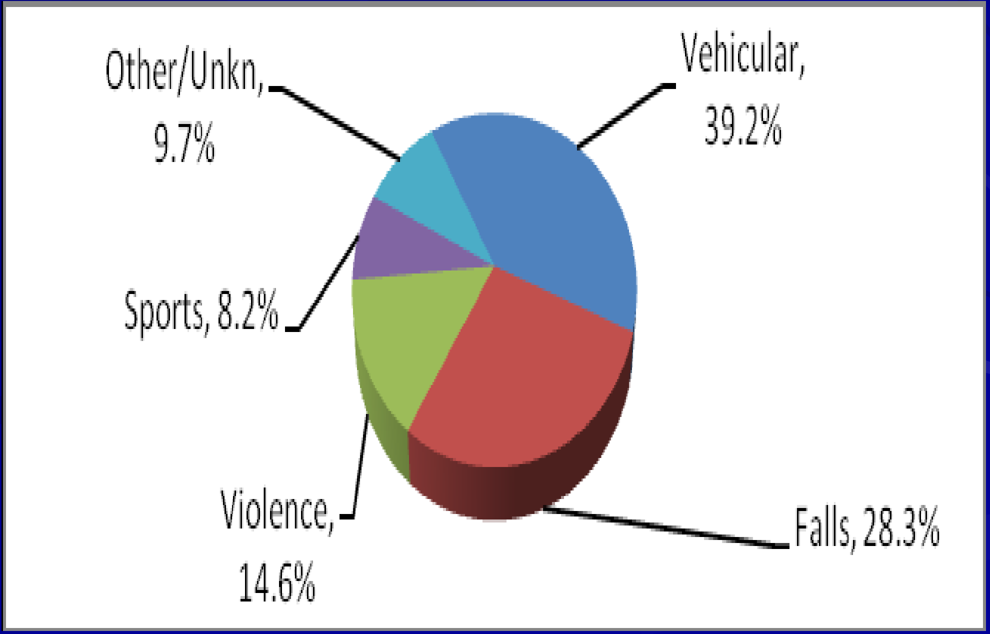
Spinal Cord Injury Statistics

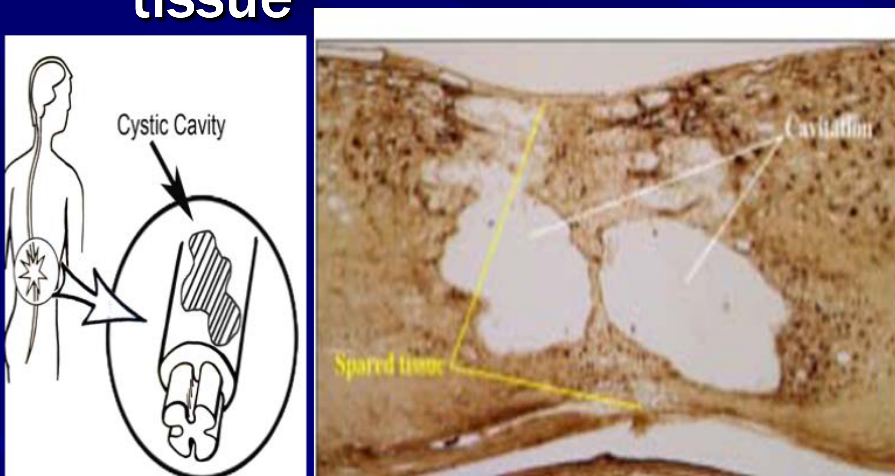
- ~270,000 people living with a SCI at a total annual cost of nearly $4 billion per year
- ~12,000 new cases each year
- A new SCI occurs every 41 min

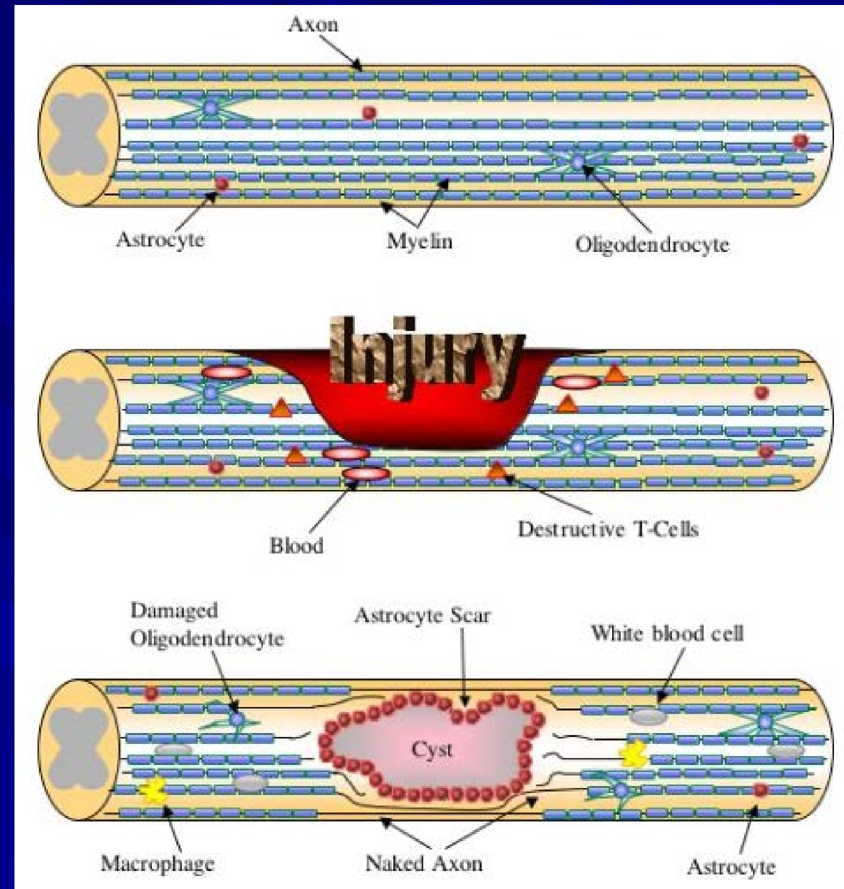


1. Silver J, Miller J (2004). Regeneration Beyond the Glial Scar. Nature Reviews Neuroscience 5: 146-156.
2. National Spinal Cord Injury Statistical Center

Syringomyelia in SCI

- Syrinx, or cyst, is not permissive to axonal re-growth
- Cyst is surrounded by inhibitory glial scar and rim of spared tissue





a) Cartoon of cystic cavity at epicenter of spinal cord lesion. b) After a contusion injury (bruising of the spinal cord) a cavity forms at the injury site with a small rim of spared tissue. Illustration from www.reeve.uci.edu

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Regenerating a spinal cord

- Step 1: provide a permissive substrate to "fill the gap"
- Step 2: make injured axons to grow through scar and beyond gap
- Step 3: make injured axons appropriately connect to targets

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"Filling the gap" with tissue-derived materials

- Transplantation of peripheral nerves
- Fetal spinal cord tissue
- Schwann cells
- Stem or progenitor cells of various origins
  - including olfactory ensheathing glia and bone marrow stromal cells
- Limited regeneration was seen, but **regeneration** was shown
- Clinical utility may be reduced by difficulty in obtaining **reliable source of tissue**

Central Hypotheses

- "Filling the gap" with a synthetic, biocompatible, growth-permissive matrix will enable regenerating axons to cross the lesion
- This research aims to develop a 3D-biodegradable regenerative matrix comprised of an aligned electrospun scaffold with a molecular orientation gradient for spatially controlled release of growth factors and agents to neutralize inhibition as a means to promote axonal repair and regeneration after SCI.

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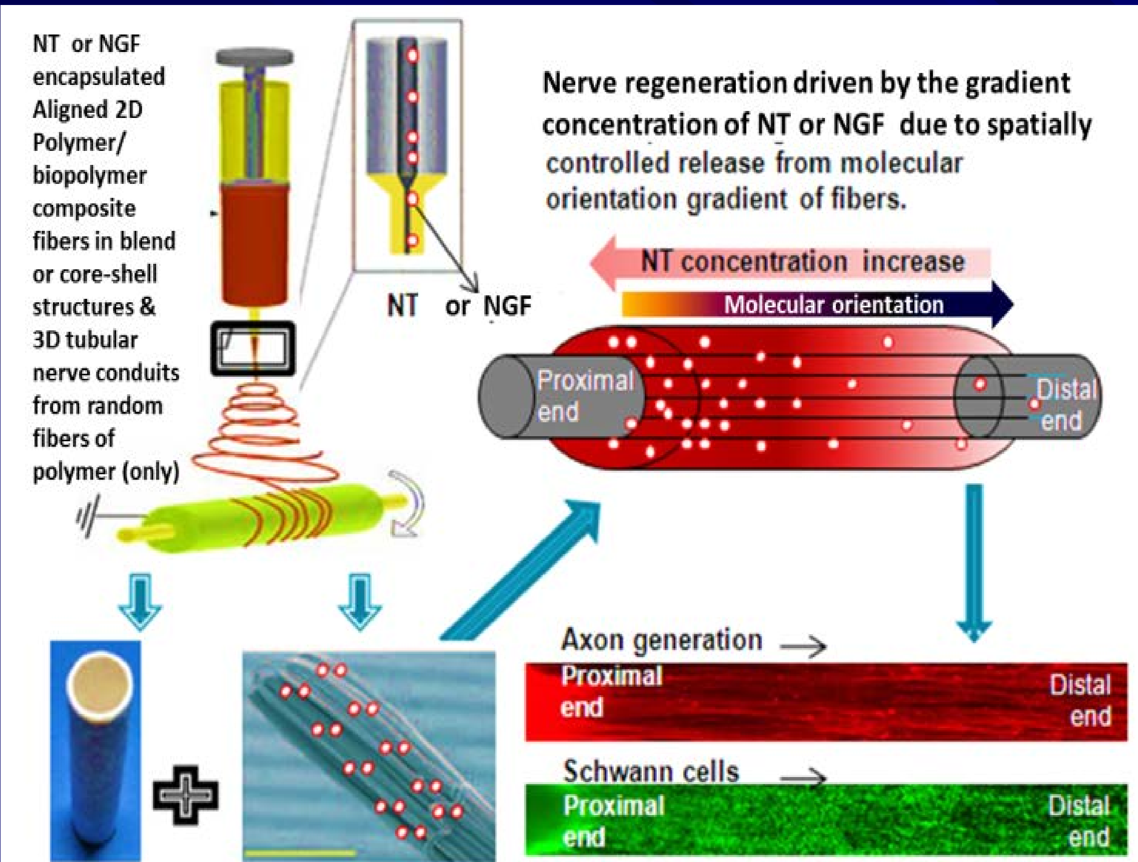


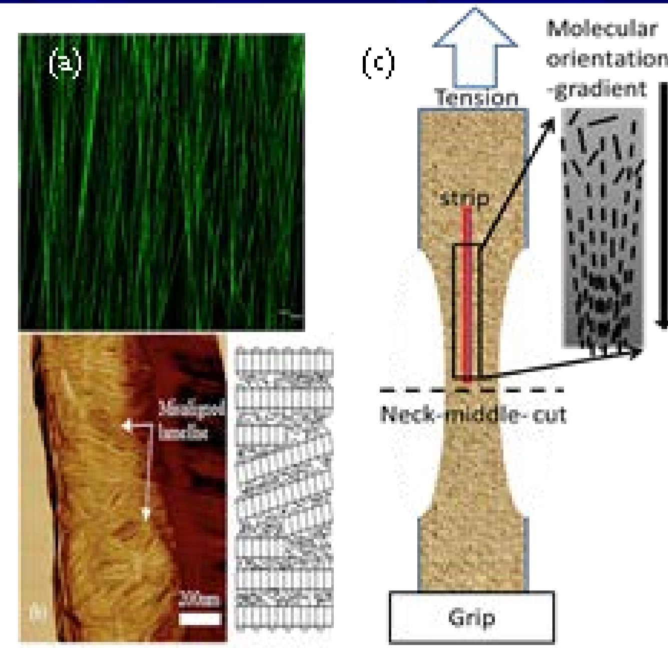


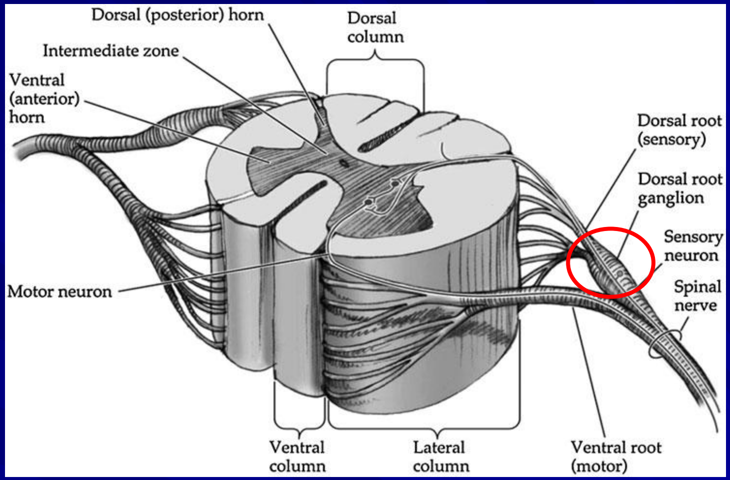
Fig 4. Preliminary data showing CLSM image of PCL/collagen 80/20) fibers (b) AFM image of miscellaneously packed lamellae in single fiber with schematic drawing. (c) Concept of uni-axial stretching will align the polymer molecules and give an orientation

Our concept of molecular orientation gradients driving the delivery of neurotrophins in a timed manner to modulate regeneration using non-isotropic nanofibrous conduits for “long” gaps’. The aligned fibrous mats encapsulated with neurotropin-3 (NT-3) or NGF will be packed in 3D tubular conduits after “uni-axially drawing until necking”.

Preliminary Work

Task # 1

- **Develop a reliable protocol for harvesting dorsal root ganglia from P1 Sprague-Dawley rat pups to grow in culture**



1) **Grow DRG explant cultures on laminin-coated coverslips and polymer scaffolds in vitro**

Preliminary Work Contd.

- **Establish a system for tracking and quantifying DRG growth in vitro using brightfield microscopy after plating**
  - How many DRGs float, how many stick, how many grow? i.e. what is our success rate?
  - Can we use all the DRGs that exhibit some growth?

DRG Growth Scale

- DRGs don’t grow uniformly
- Developing a scale for judging each DRG based on: Growth, Length, and Interconnectedness of neurites

Growth

- Judge 2 Aspects
  - Even/Uneven Growth distribution
  - Some/Extensive Growth thicknes

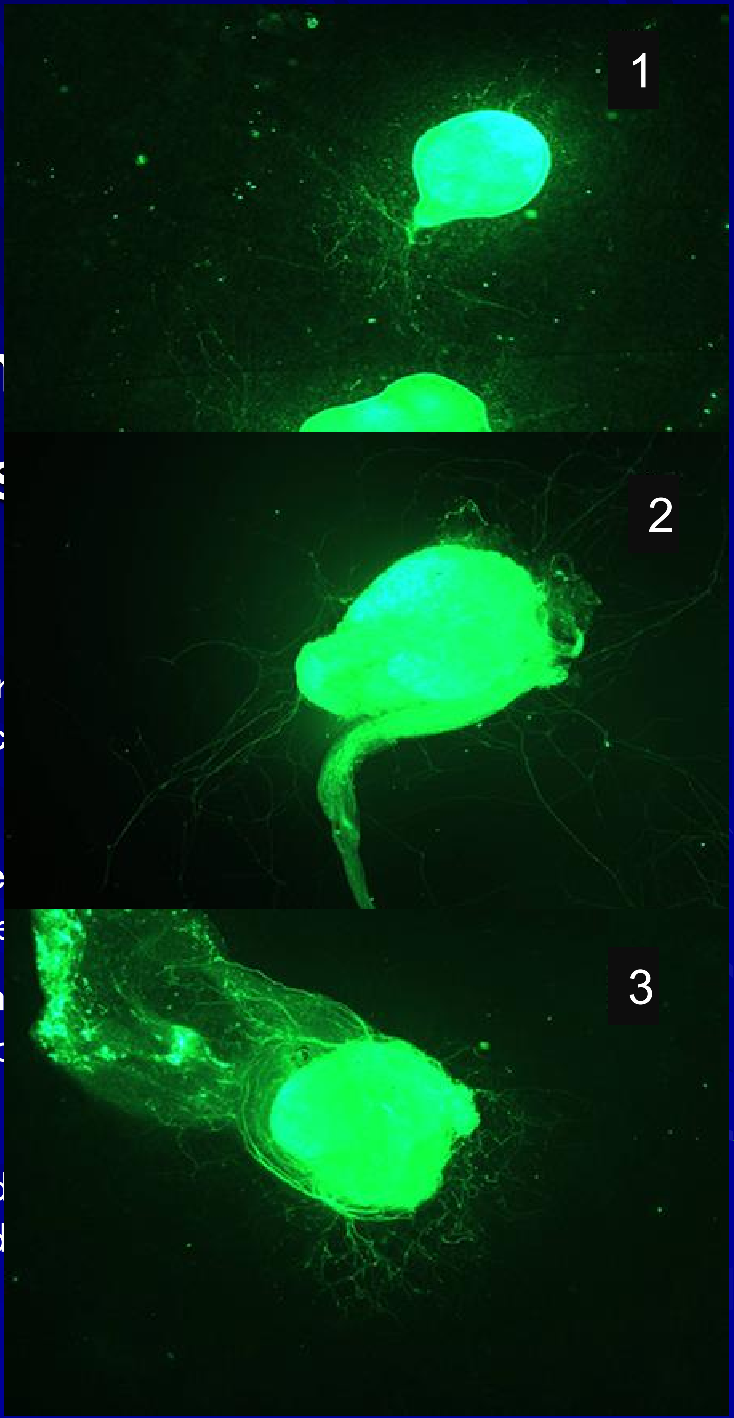
0 – None – no neurites observed

1 – Some Uneven – neurites observed but distributed unevenly ar the perimeter and sparsely enough that most neurites can be trac easily

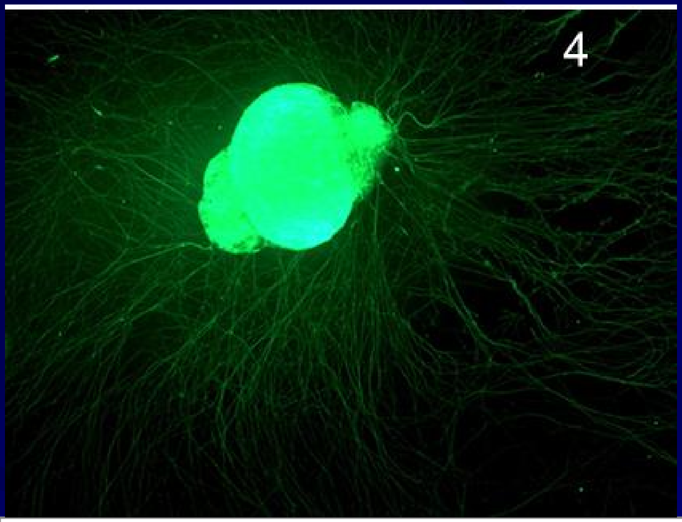
2 – Some Even – neurites observed distributed evenly around the perimeter and sparsely enough that most neurites can be traced e

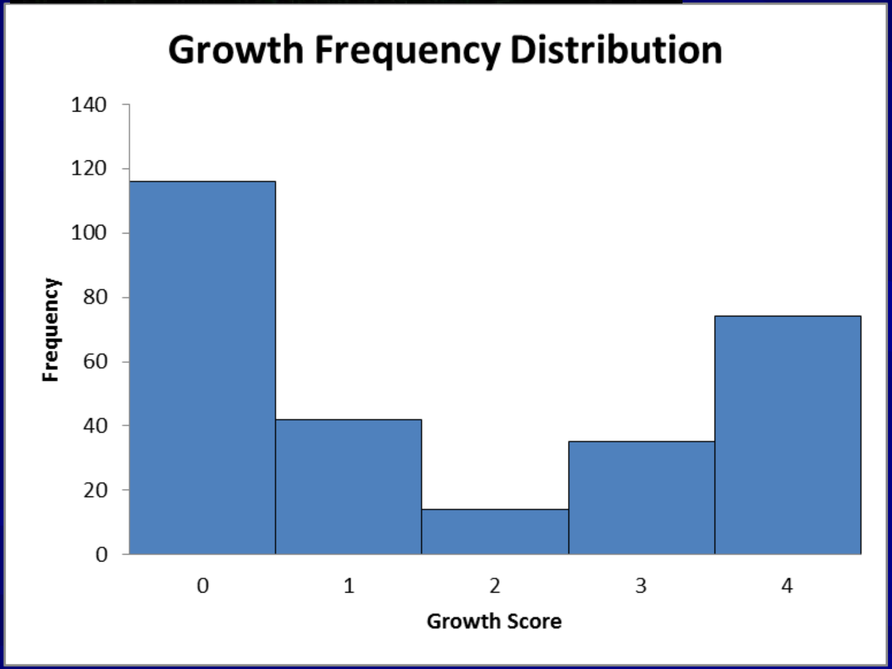
3 – Extensive Uneven – neurites observed but distributed uneven around the perimeter and thickly enough that most neurites cannc traced easily

4 – Extensive Even – neurites observed distributed evenly around perimeter and thickly enough that most neurites cannot be traced

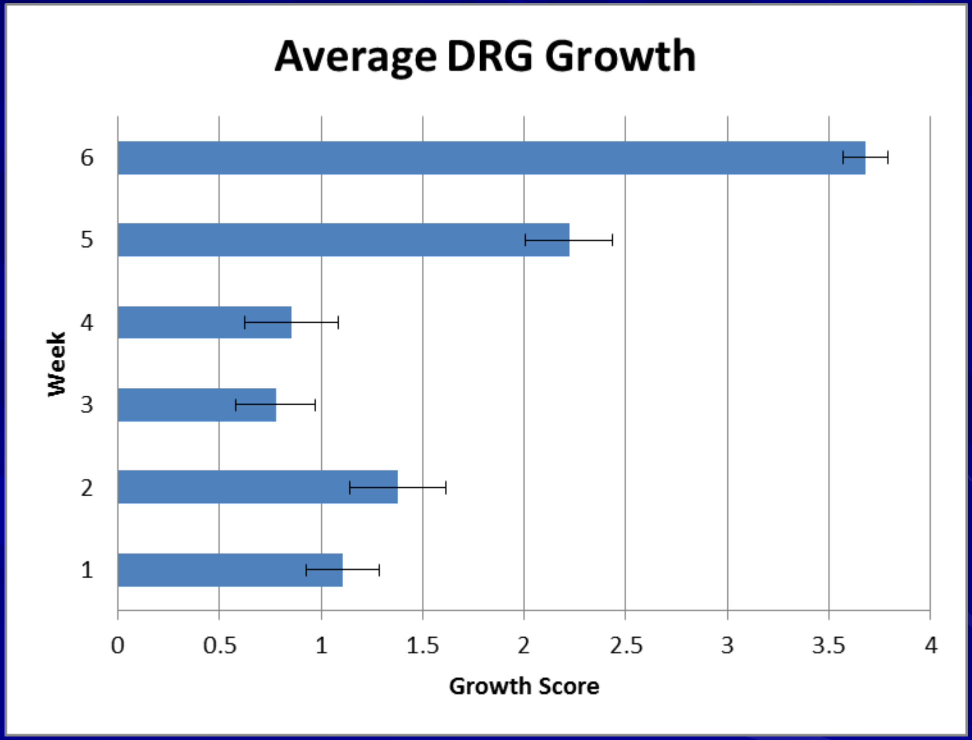


Summary of Growth Condition Analysis





~300 DRGs were observed and ranked



These data indicate the improvement in growth with optimization of culture conditions over time. Each week is one separate dissection/preparation.

Length

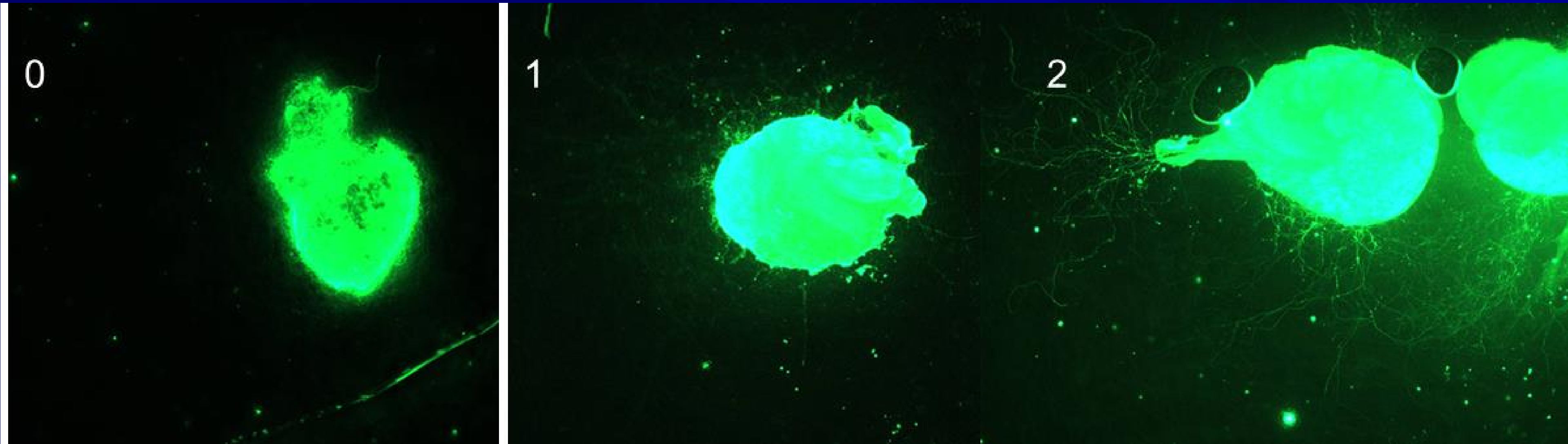
- **Take an average of the lengths of the neurites and compare it to the radius**

0 – None – not growing

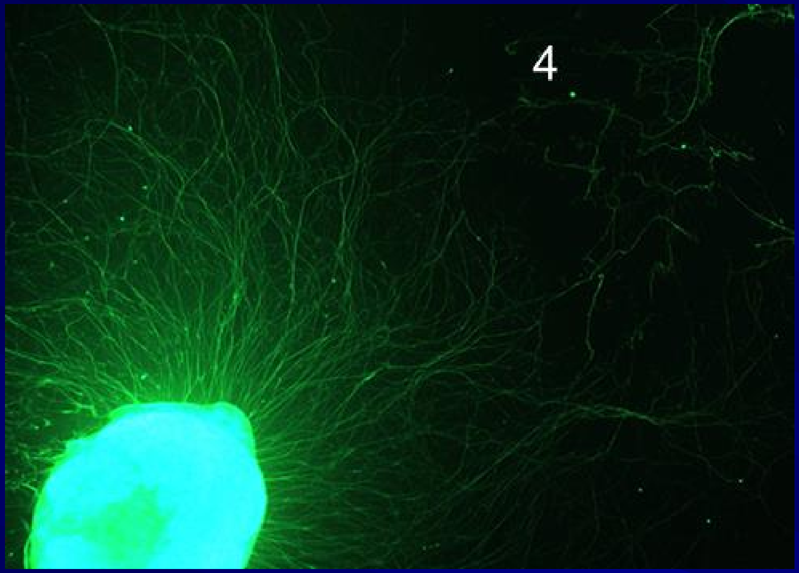
1 – Short– less than the length of the DRG radius

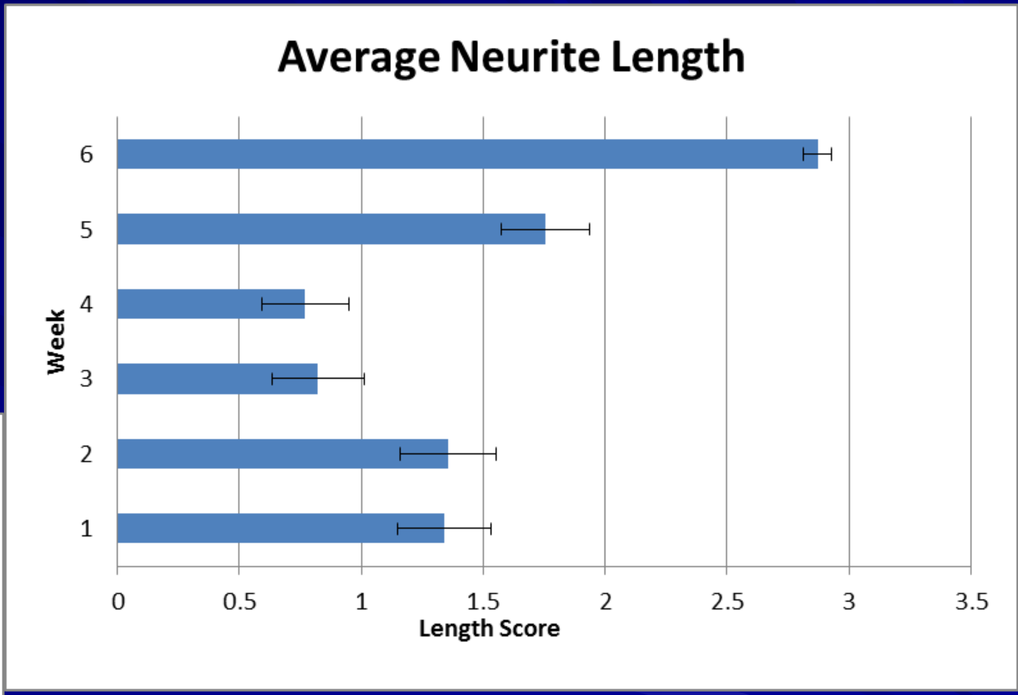
2 – Moderate– less than 1.5 times the length of the radius

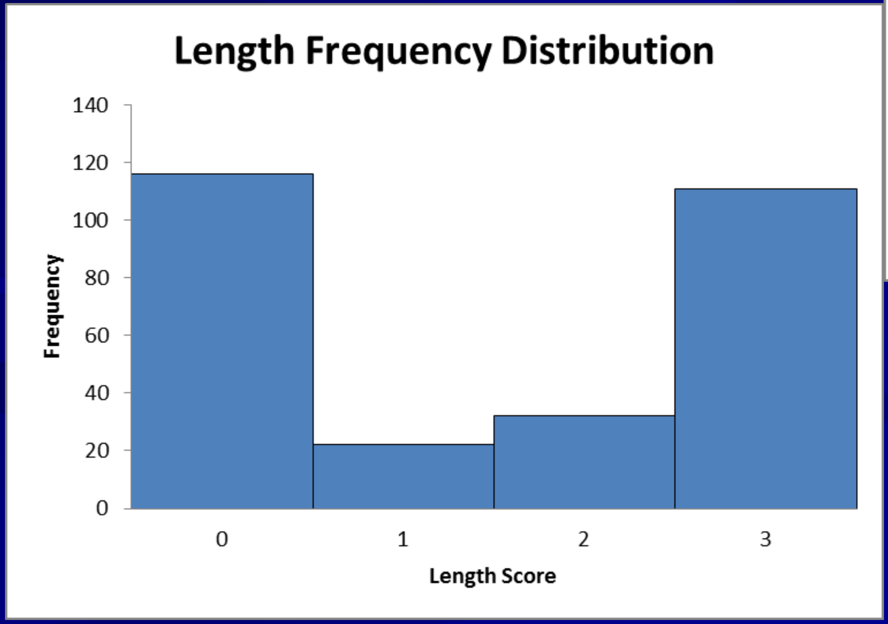
3 – Extensive– greater than 1.5 times the length of the radius



Summary of Length

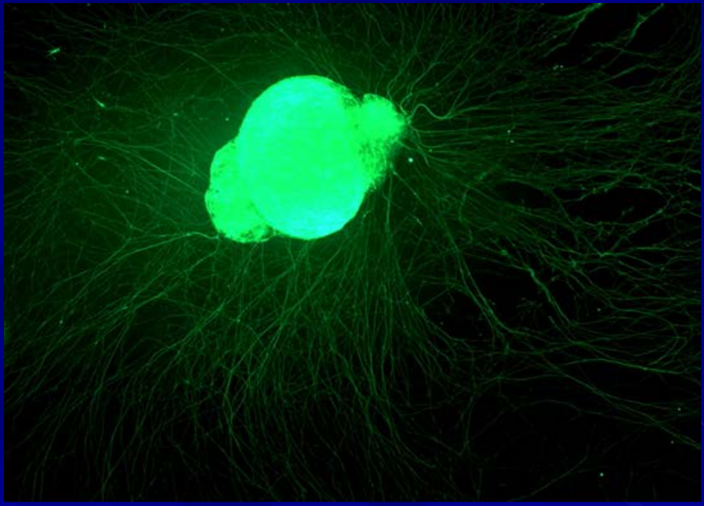


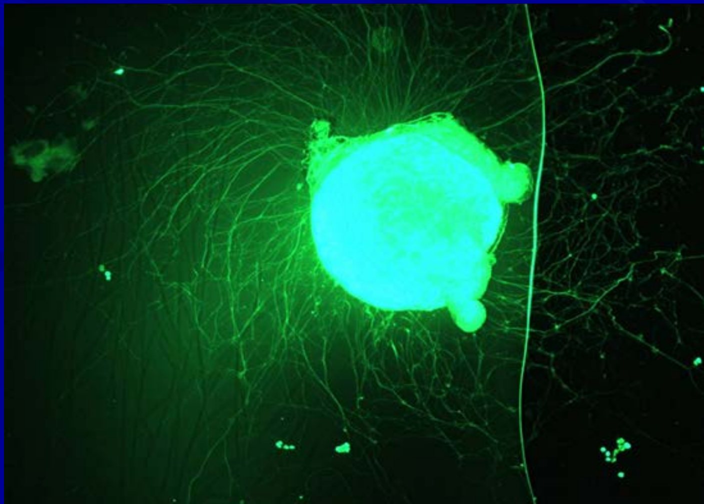




Interconnectedness

- Distinguish between DRGs with neurites growing in parallel and those growing in an interconnected network
- Most difficult category to judge





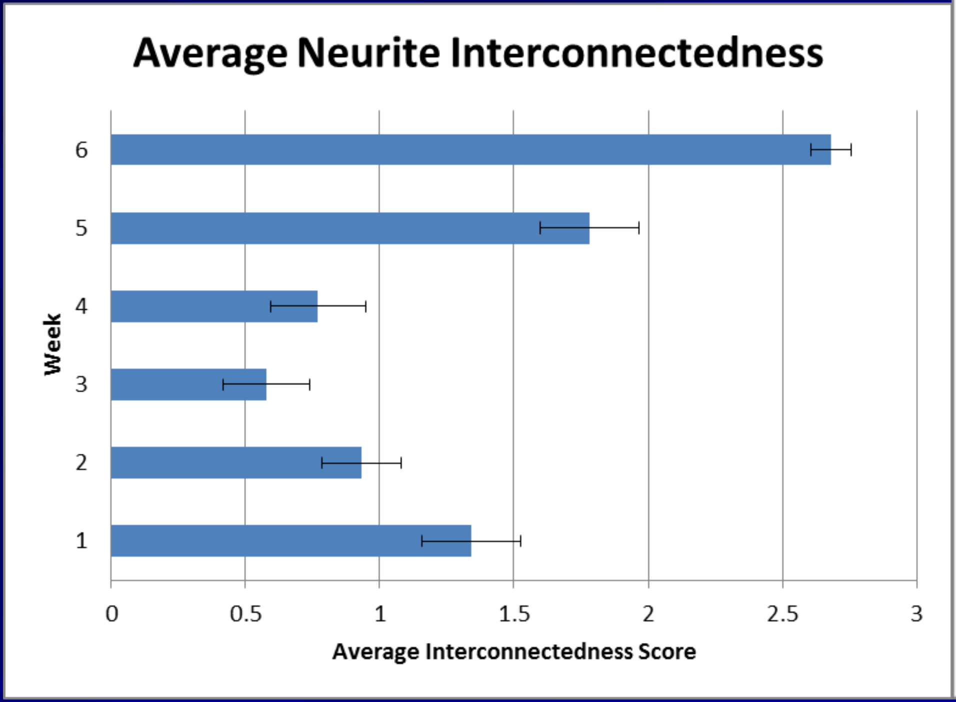
0- None- >95% of all neurites grow in parallel without connecting and creating nodes

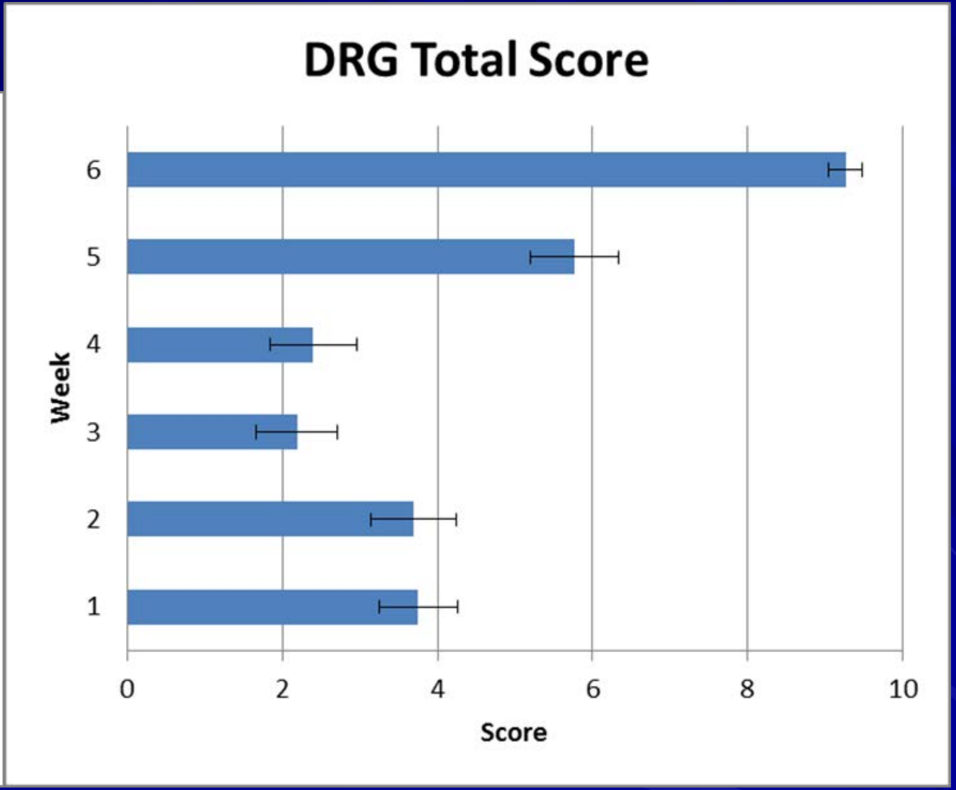
1- Mild- 80-95% of all neurites grow in parallel without connecting and creating nodes, remaining neurites are more likely to intersect repeatedly

2- Moderate- 30-80% of neurites grow in parallel without connecting and creating nodes, remaining neurites are more likely to intersect repeatedly

3- Extensive- <30 % of the neurites grow in parallel without connecting and creating nodes, the remaining neurites are more likely to intersect repeatedly

Summary of Total Scale Ranking



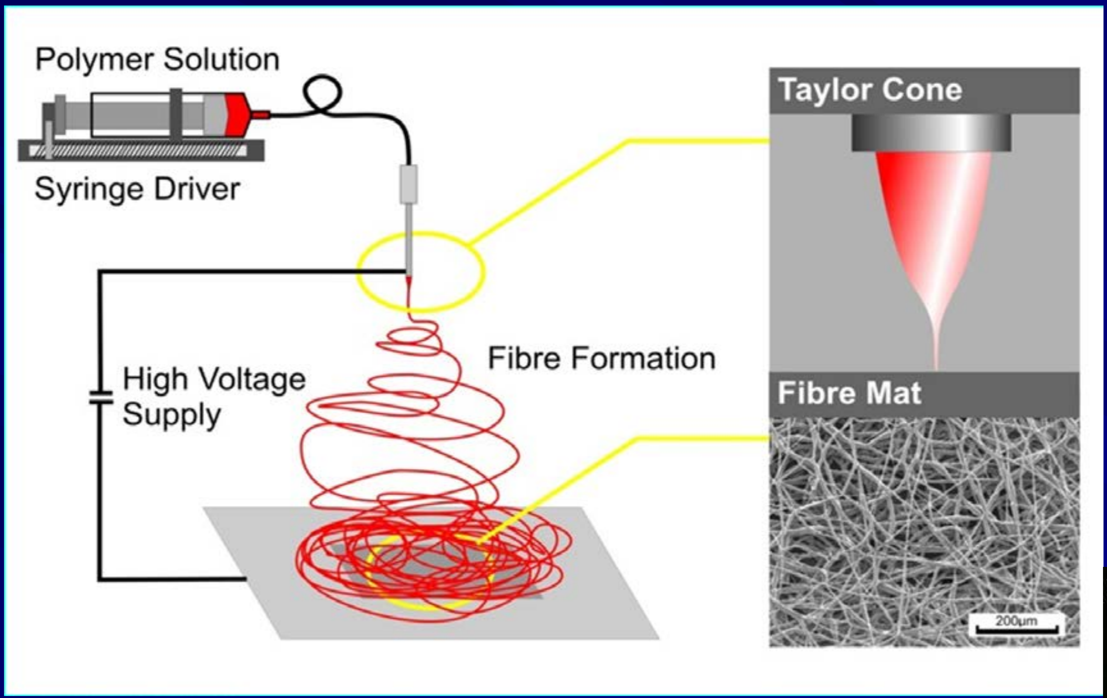


We have optimized our DRG explant culture conditions/skills (in terms of growth, length and interconnectedness score)

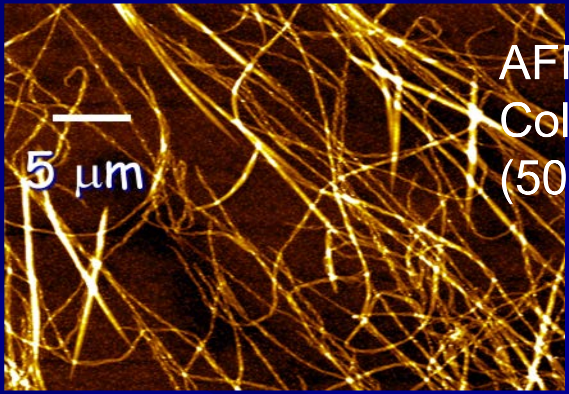
This semi-quantitative scale could be used for a rapid screening of biomaterials

Progress- Nanomatrix Fabrication

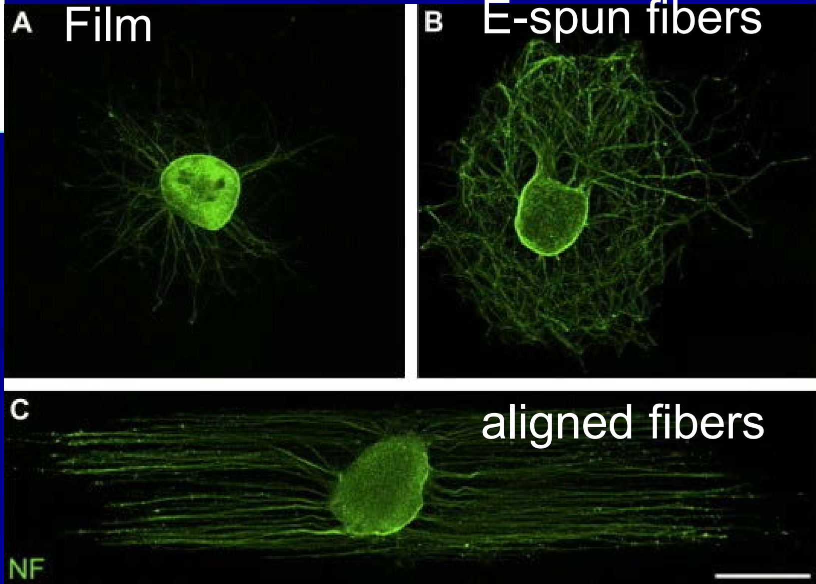
Task # 2



Large surface area/volume
Biomimetic morphology (nanofibers)
Inter-connected pore



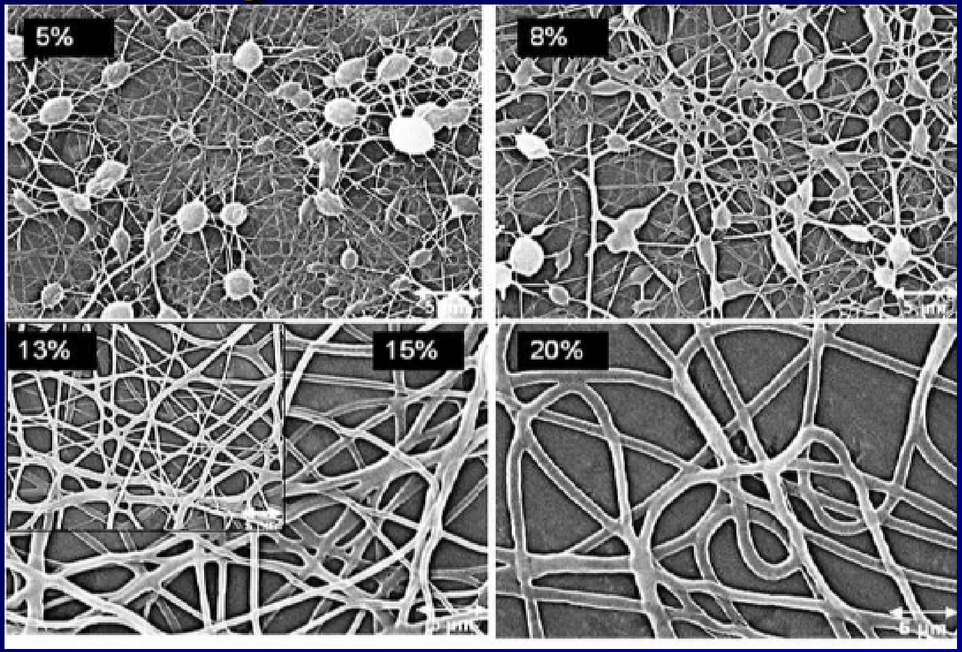
AFM
Collagen
(50-500 nm)



Parameters spinnability & fiber diameter

1. Concentration (viscosity)
2. Flow rate of solution
3. Electric potential
4. Collector distance & geometry

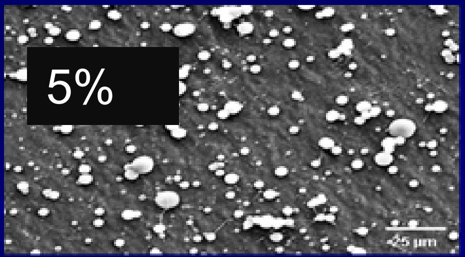
Polymers   PDO and SPU

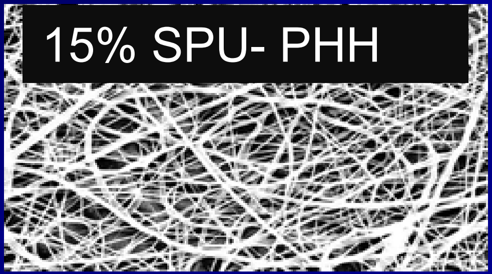


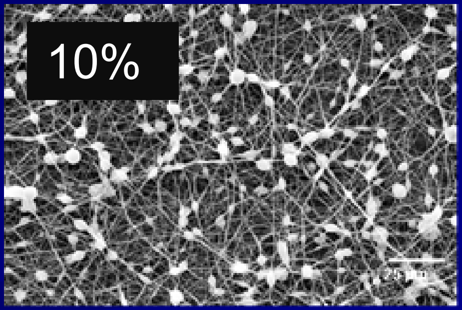
Biocompatible
Biodegradable

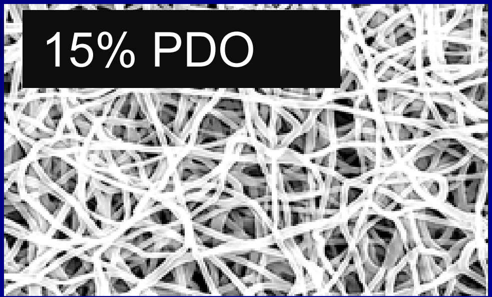
PDO is semicrystalline with elastic modulus 7.1 MPa (e-spun fibrous)

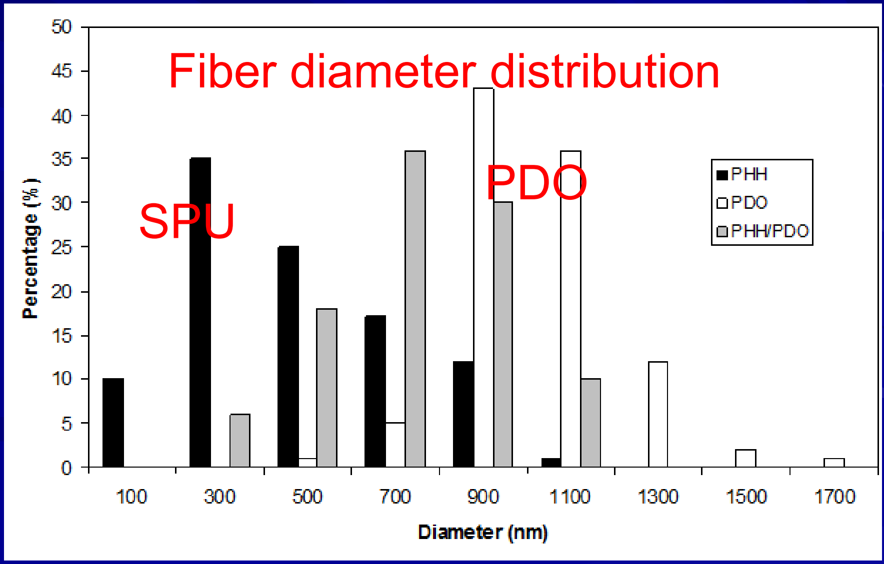
SPU (PHH) is elastomeric with modulus 0.58MPa (close to spinal cord tissue- 200-600 kPa)











Optimization of Immunohistochemistry

- Needed consistent labeling of fine process with clear delineations of neurites
- Evaluated several antibodies against neurofilament (light, medium and heavy chain)

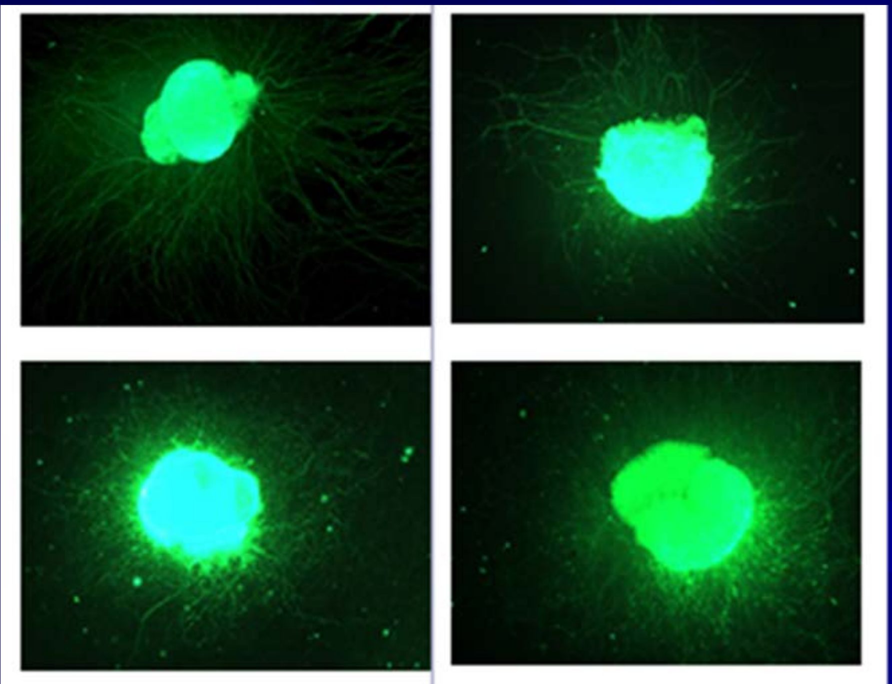


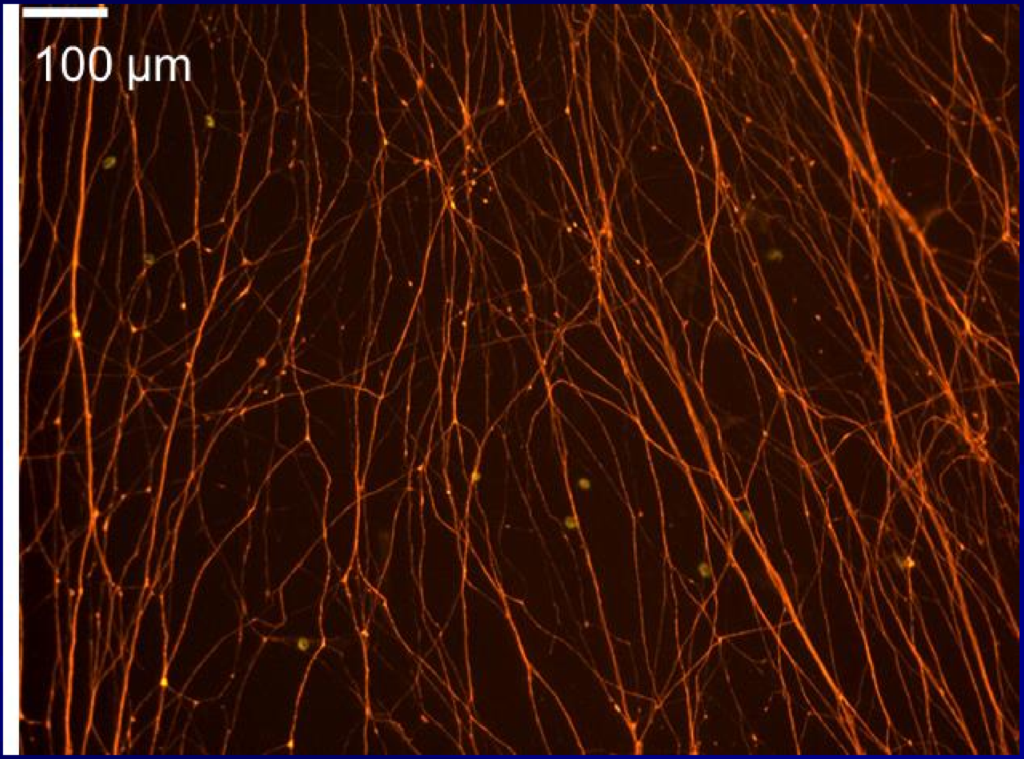
Figure DRG explants demonstrate comparable growth at 3 days on (a) & (b) laminin coated glass coverslips and (c) &(d) electrospun PDO scaffold

Glia-fibrilary acid protein (GFAP) stained

Supplier	Antibody	Host	Low	High
Millipore	NF H&M	M	1:100	1:5000
Millipore	**NF H**	**Ch**	**1:200**	**1:1000**
Millipore	NF L	M	1:100	1:10000
Cell Signaling	NF M (RMO)	M	1:100	1:1000
Cell Signaling	NF L (DA2)	M	1:100	1:1000
Cell Signaling	NF L-R	R	1:100	1:1000
Cell Signaling	NF H (RMdO)	M	1:100	1:1000

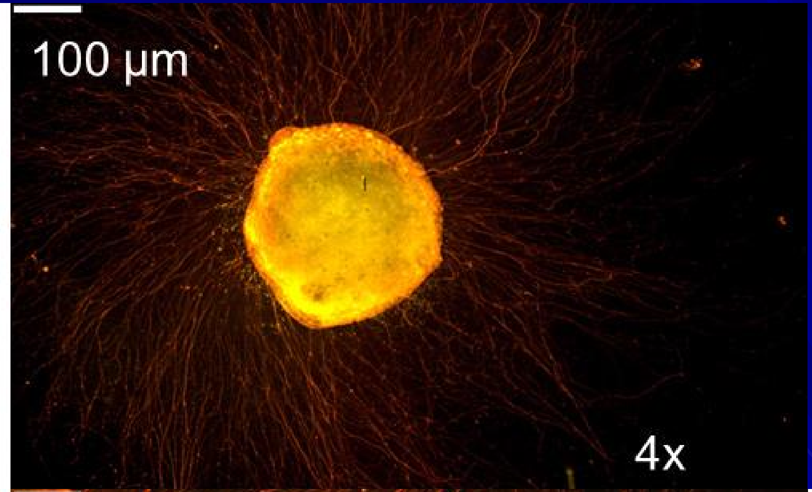
Sample of DRG with immunolabeling for neurofilament heavy chain

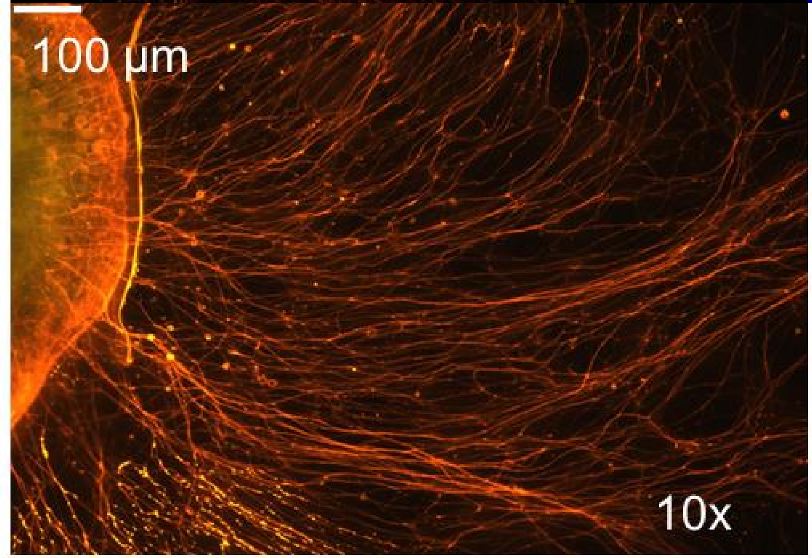
High Magnification



40x

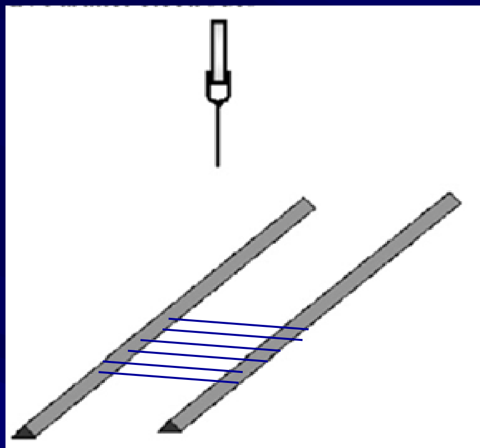
Low Magnification



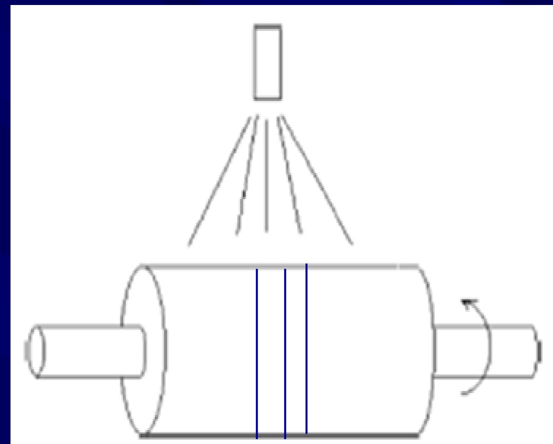


Task #3

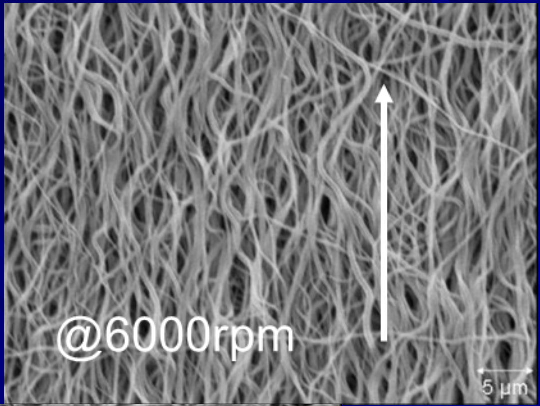
Optimization-fibers

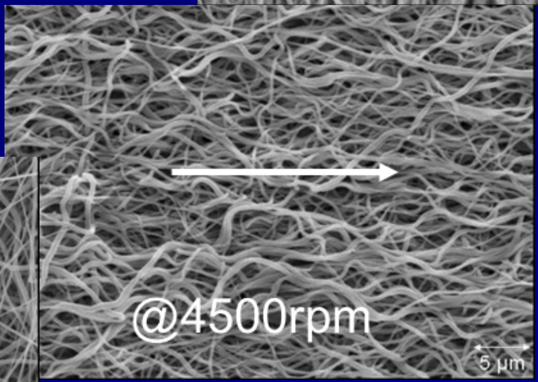


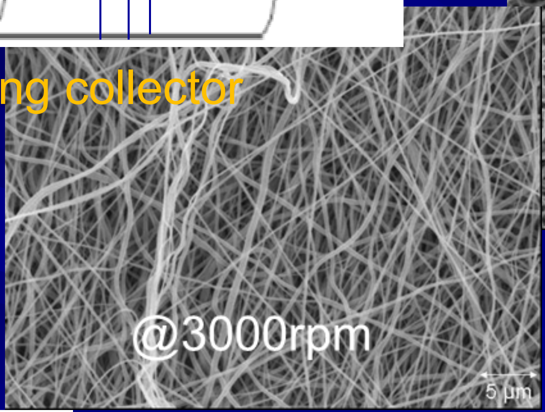
Parallel plate



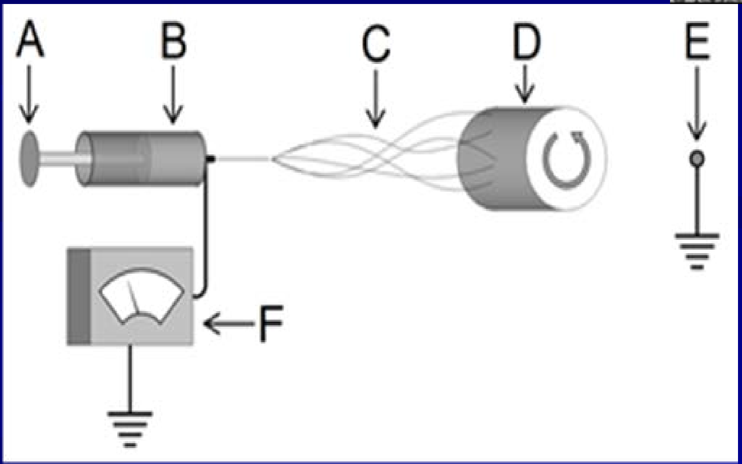
Rotating collector

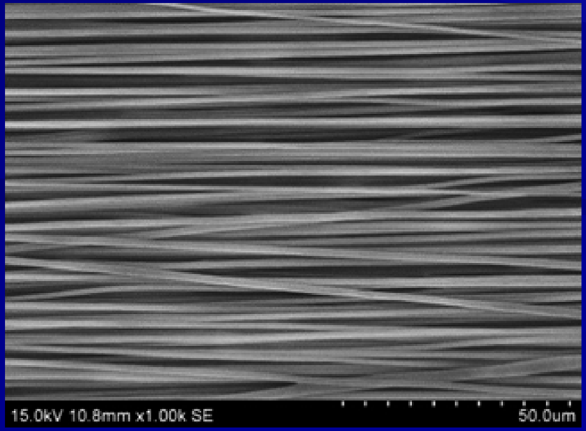


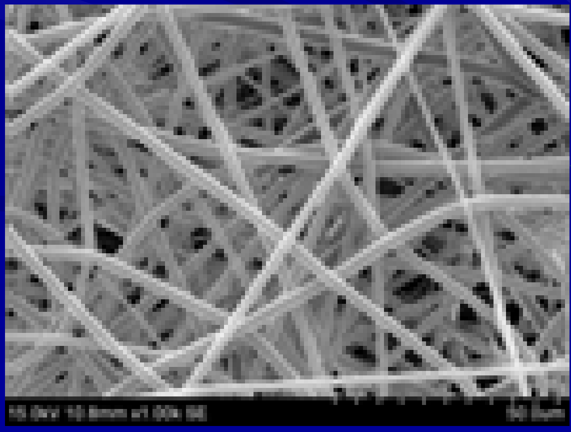




Rotating collector with







Progress & Future studies

- Have optimized the extraction and growth of rat DRG explant assay
  - Optimized growth conditions
  - Optimized immunocytochemistry visualization of neurites
- Have developed an objective, reliable, and reproducible scoring system for rapidly assessing DRG outgrowth on many substrates
- Have optimized the fiber-spinning parameters for PDO and SPU & characterized the perfect-aligned morphology and mechanical properties.

- Future studies:

(1) Fabricate and characterize orientation-gradient aligned fibers with and without NGF using optimized spinning conditions & release kinetics

(2) Compare the growth of DRG on these matrices using the growth scale and neurite tracking software (after NF H staining)

Achievements /future funds

- An abstract submitted for presenting the work during the 31st Annual National Neurotrauma Symposium (Aug 4-7, 2013), Nashville, TN.
- An IP/ provisional patent application on orientation-graded matrix for CNS regeneration has been filed.
- The preliminary data will be used to submit a collaborative R01 grant proposal to VA Merit System in December 2013 cycle.

Thank you

Growth	Avg Neurite Length	Interconnectedness
0 N	0 N	0 N
1 S/U	1 S	1 Mi
2 S/E	2 M	2 Mo
3 E/U	3 Ex	3 Ex
4 E/E		

360 Growth

None– no neurites observed

Some Uneven– neurites observed but distrbuted unevenly around the perimeter and sparsely enough that most neurites can be traced easily

Some Even– neurites observed distrbuted evenly around the perimeter and sparsely enough that most neurites can be traced easily

Extensive Uneven– neurites observed but distrbuted unevenly around the perimeter and thickly enough that most neurites cannot be traced easily

Extensive Even– neurites observed distrbuted evenly around the perimeter and thickly enough that most neurites cannot be traced easily

Avg Neurite Length

None– not growing

Short– less than the length of the DRG radius

Moderate– less than 1.5 times the length of the radius

Extensive– greater than 1.5 times the length of the radius

Interconnectedness

None– >95% of all neurites grow in parallel without connecting and creating nodes

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