UNIVERSITY of VIRGINIA
ORTHOPAEDIC SURGERY

Re-Innervation of the Neuromuscular Junction

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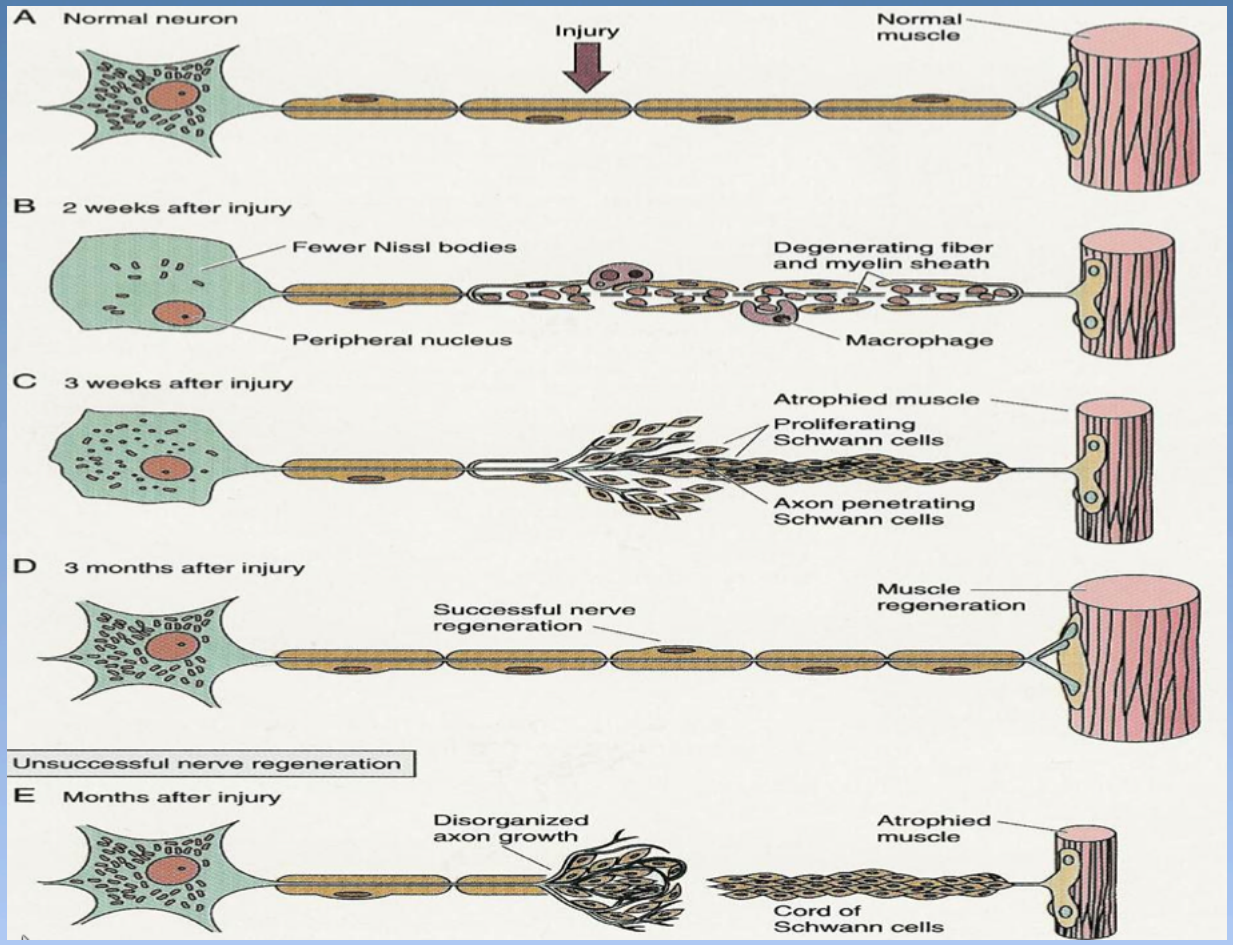
Nerve Injury



The Neuromuscular Junction

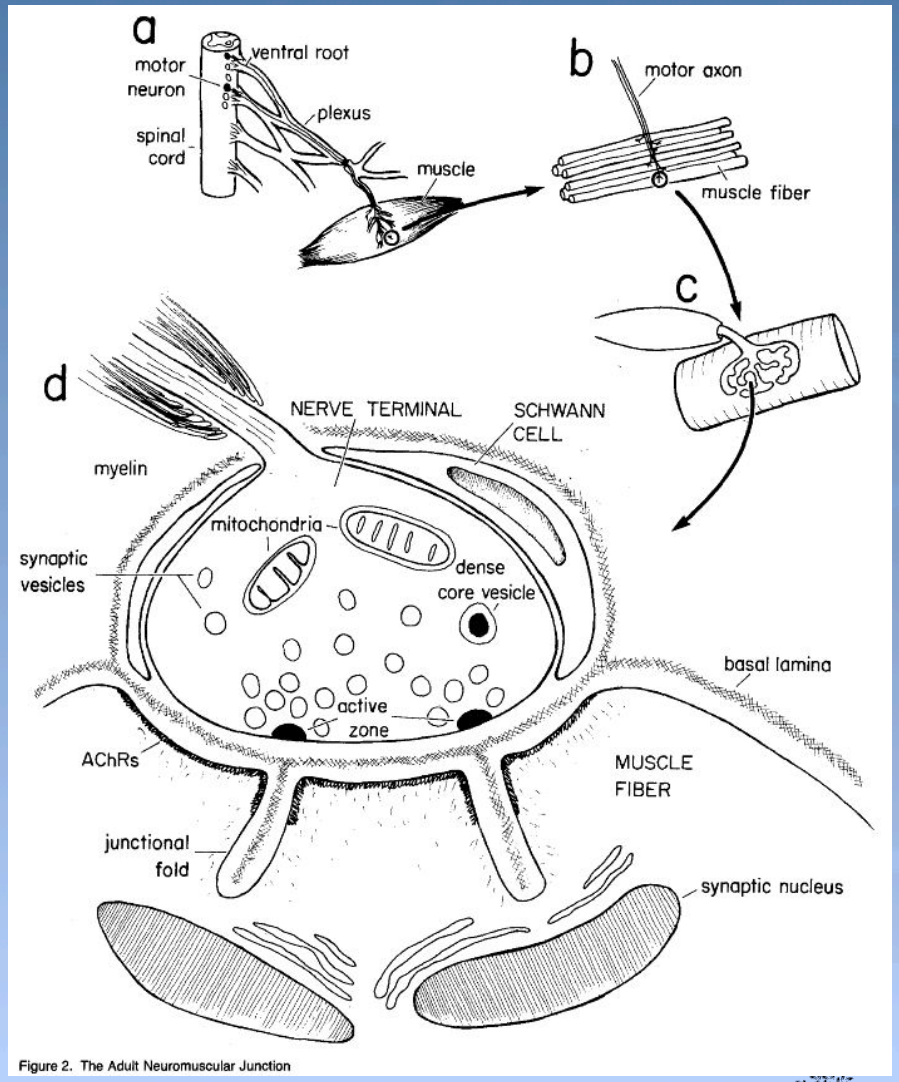
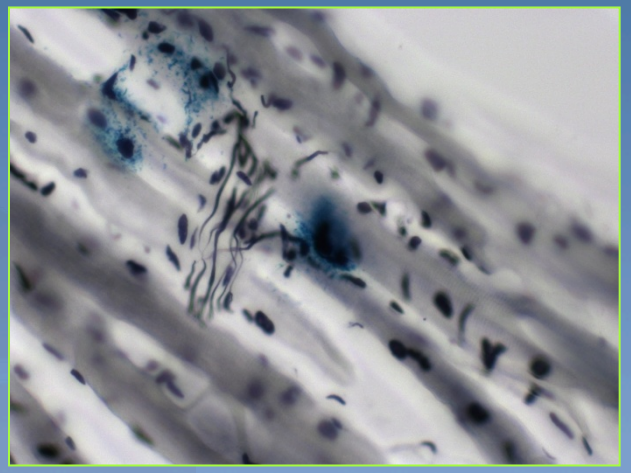
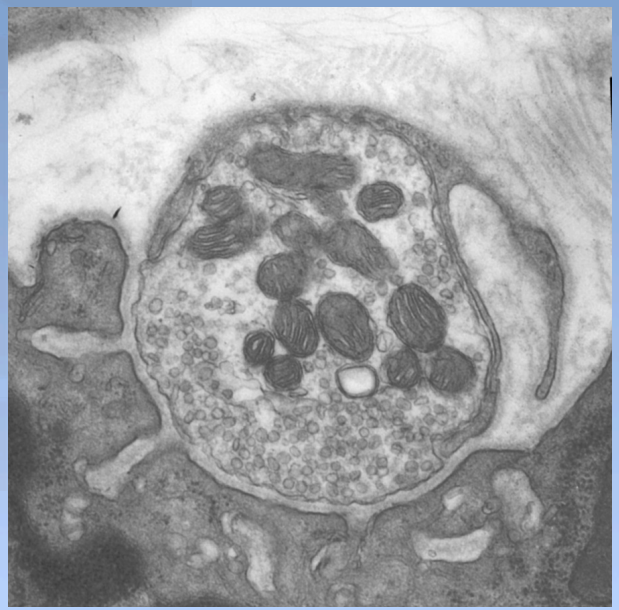
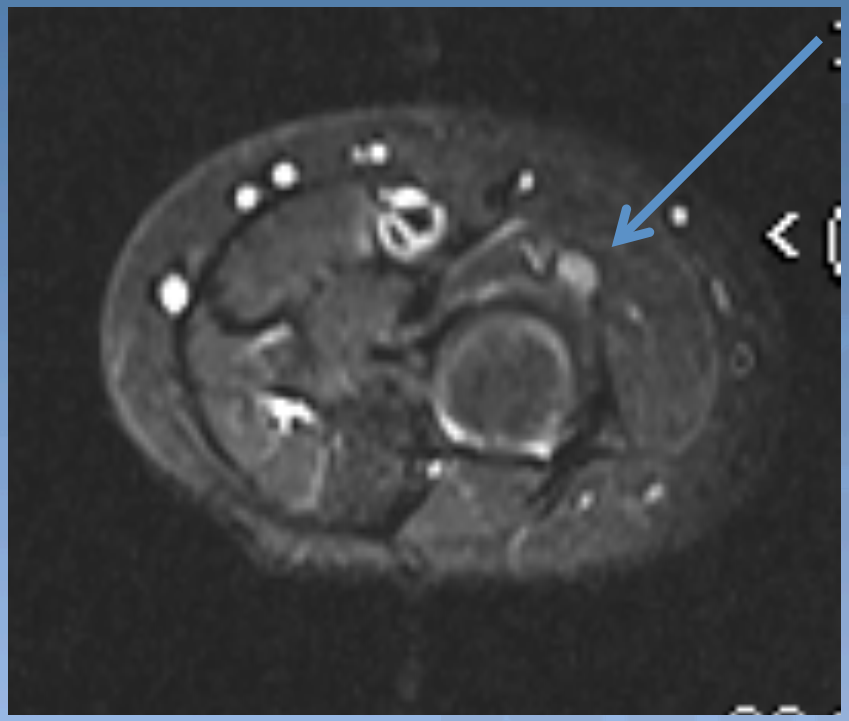


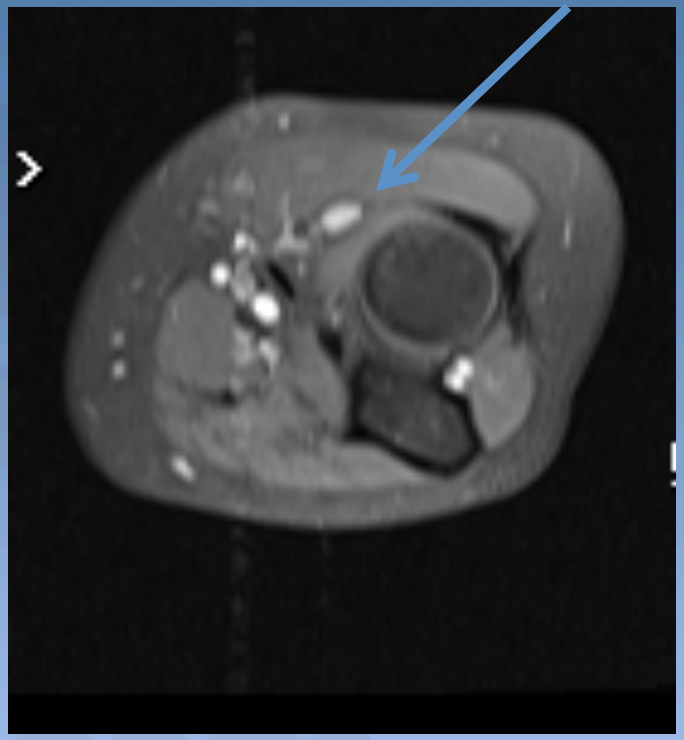
Figure 2. The Adult Neuromuscular Junction



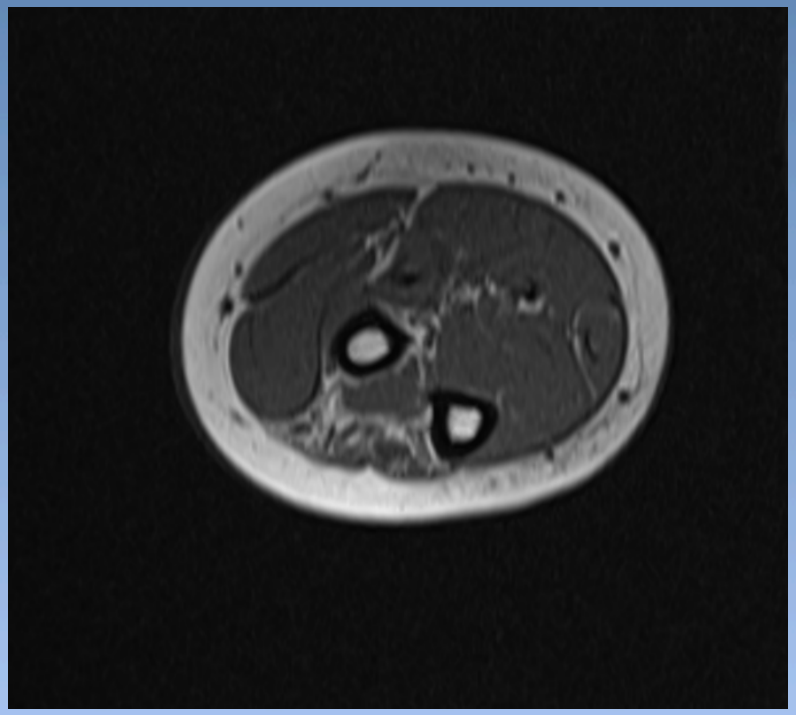


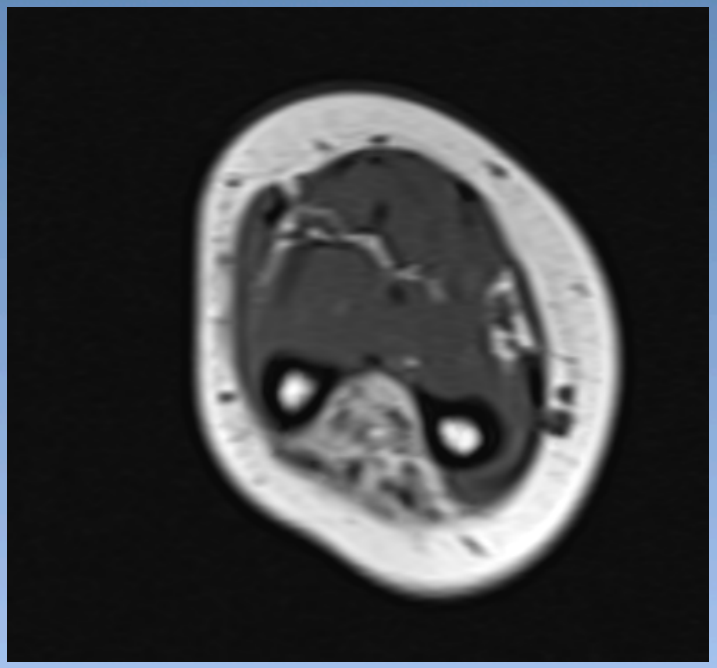
The Clinical Problem



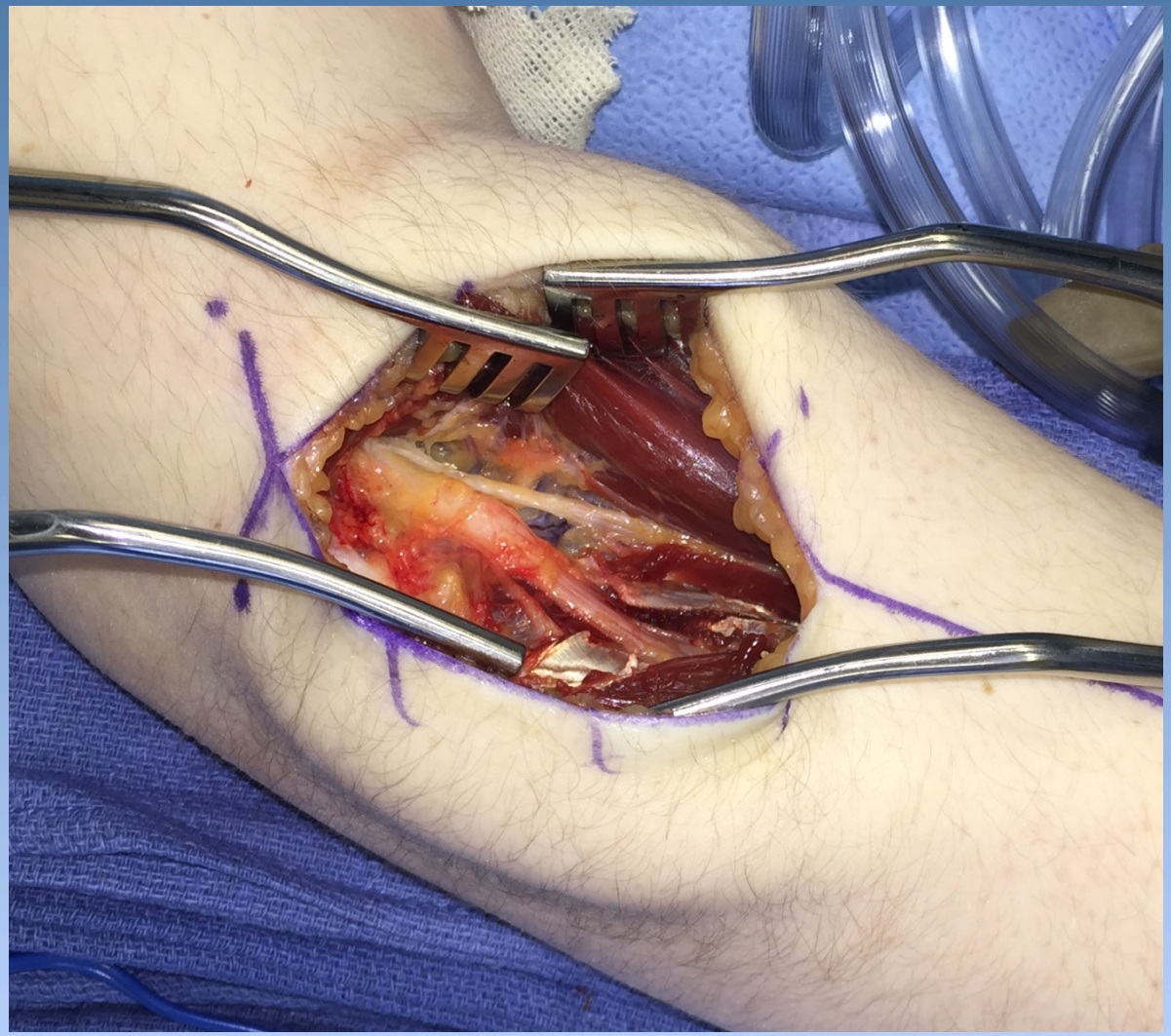


The Clinical Problem

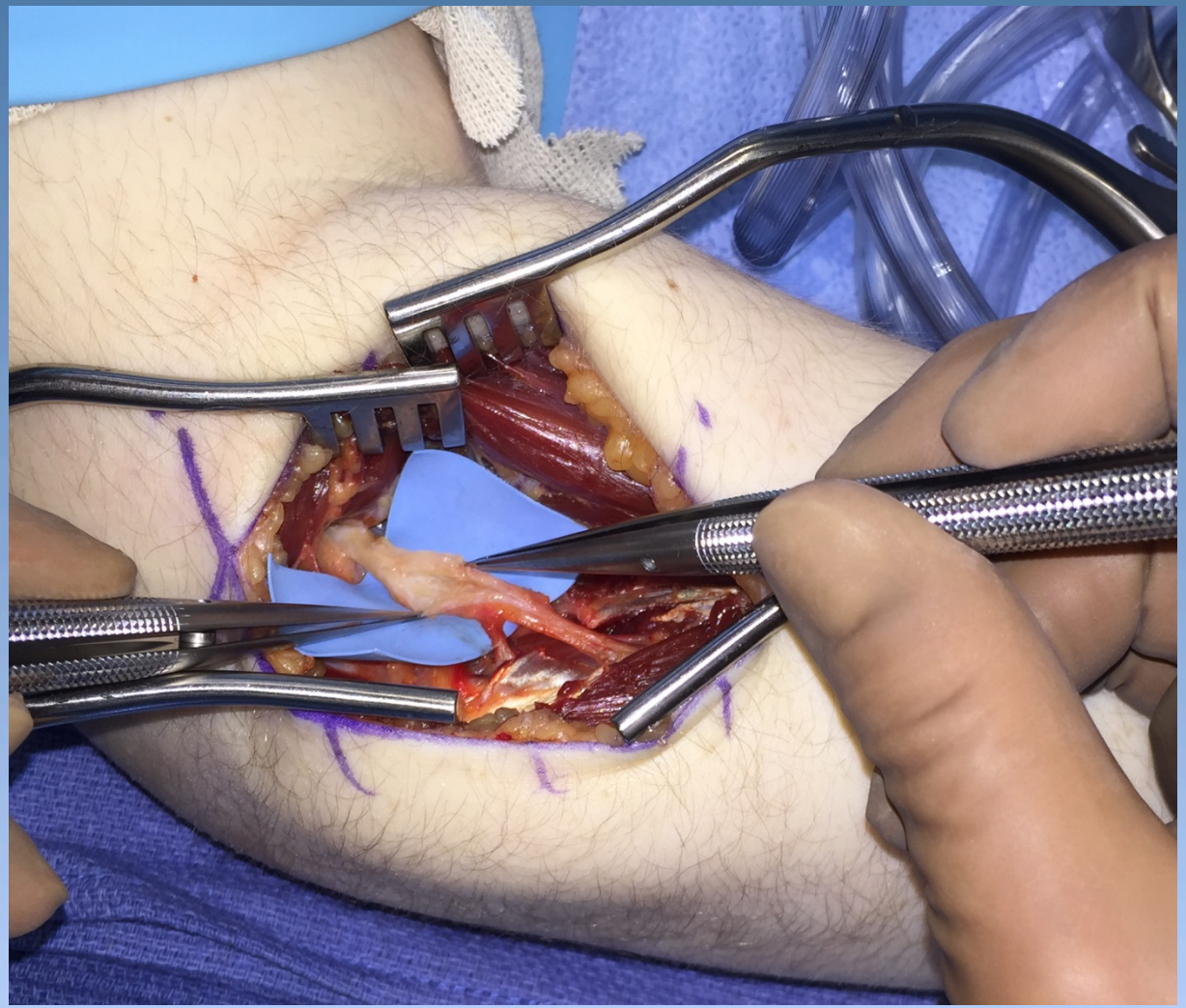




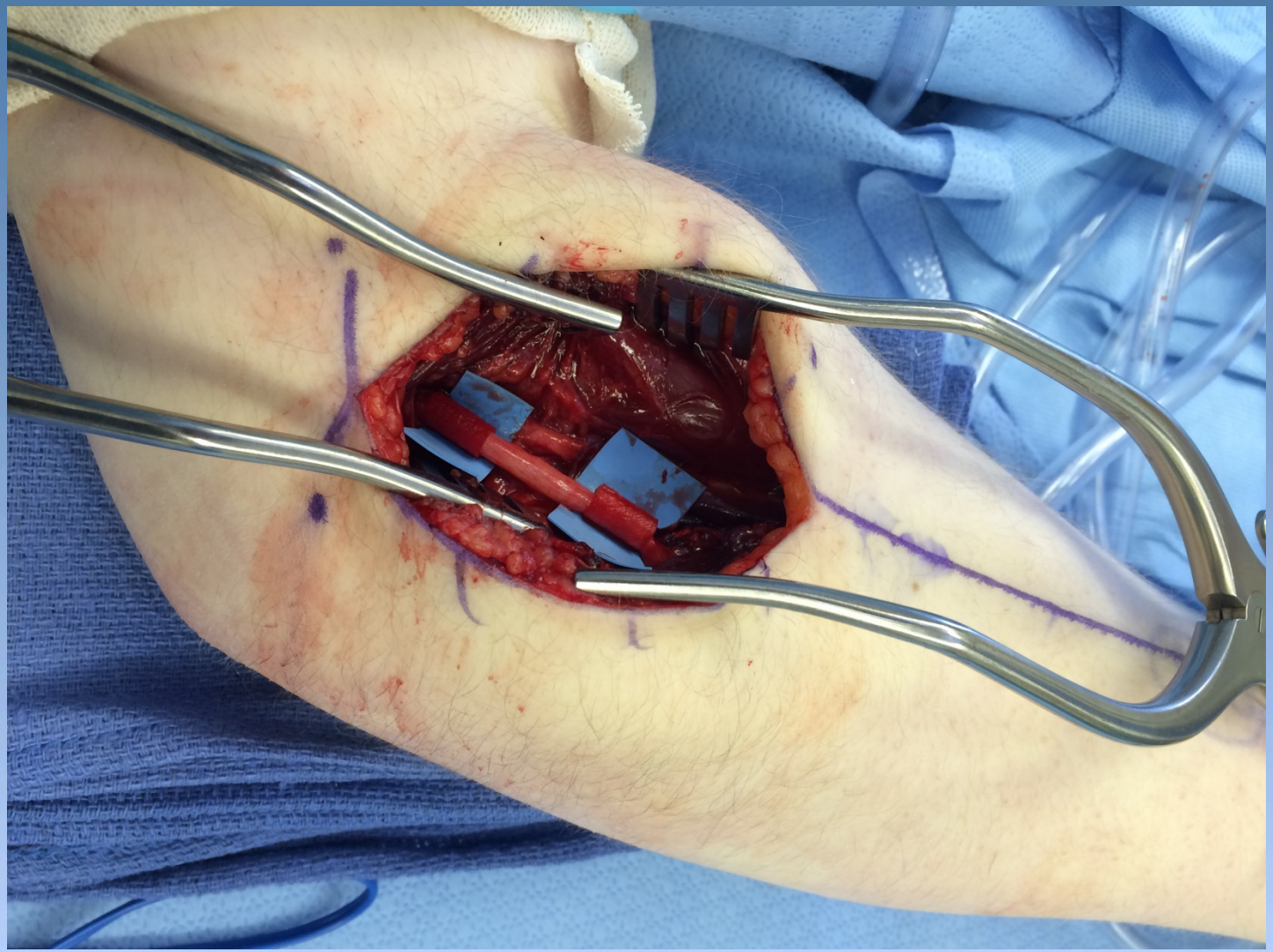
The Clinical Problem



The Clinical Problem

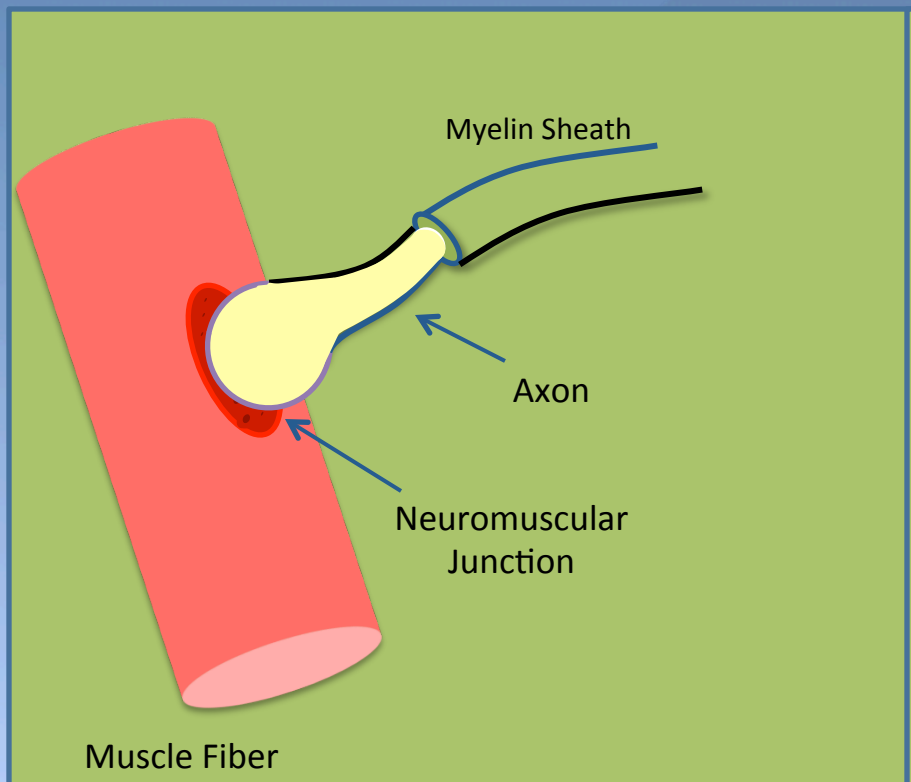


The Clinical Problem

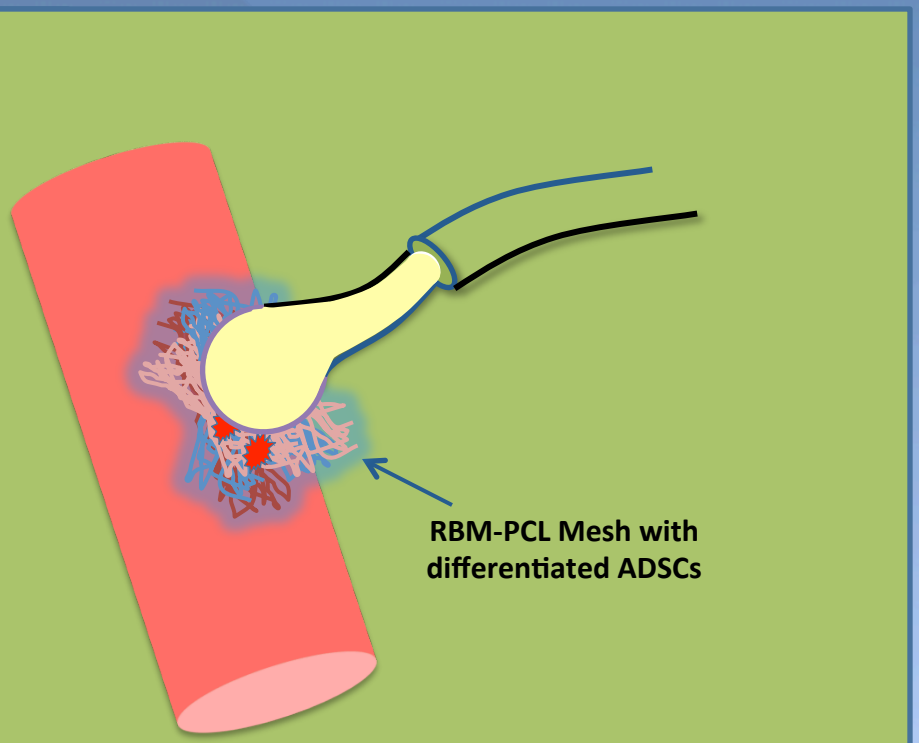


Objective

- Goal: Develop a nanofiber matrix to reconnect lacerated or avulsed nerves to skeletal muscle and prevent involution of the neuromuscular junction while recovery occurs



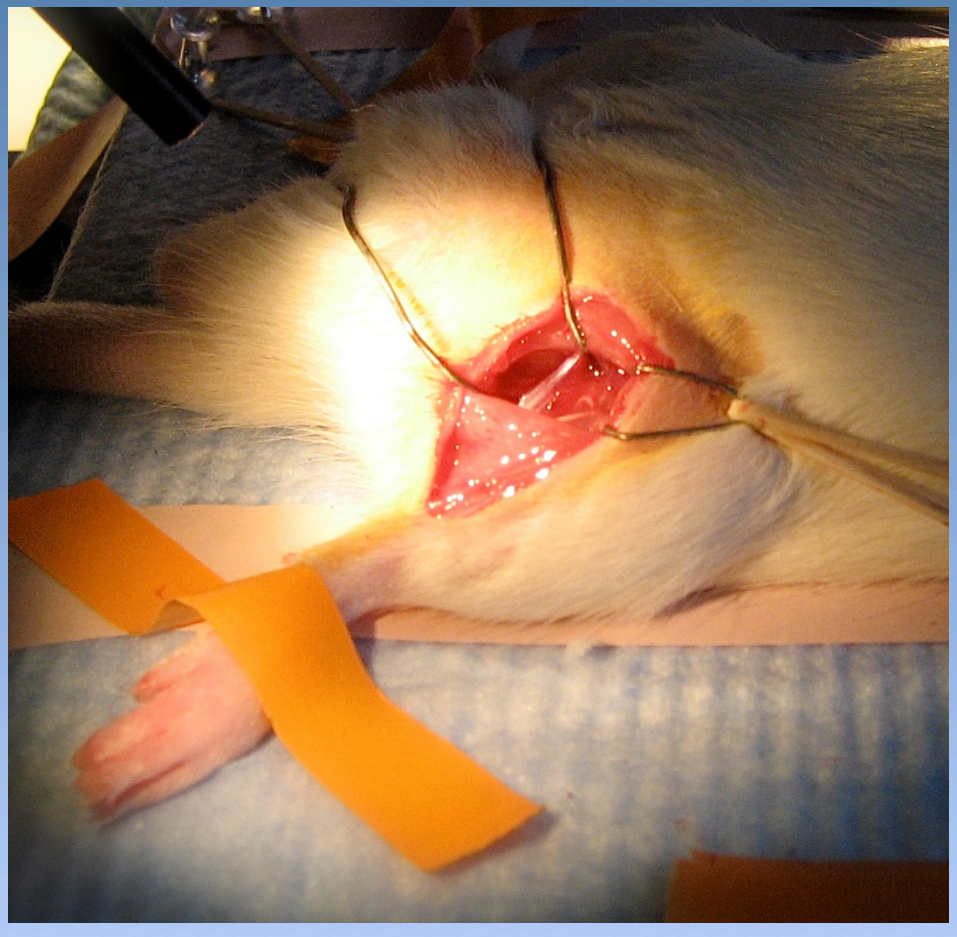
Delay in regeneration

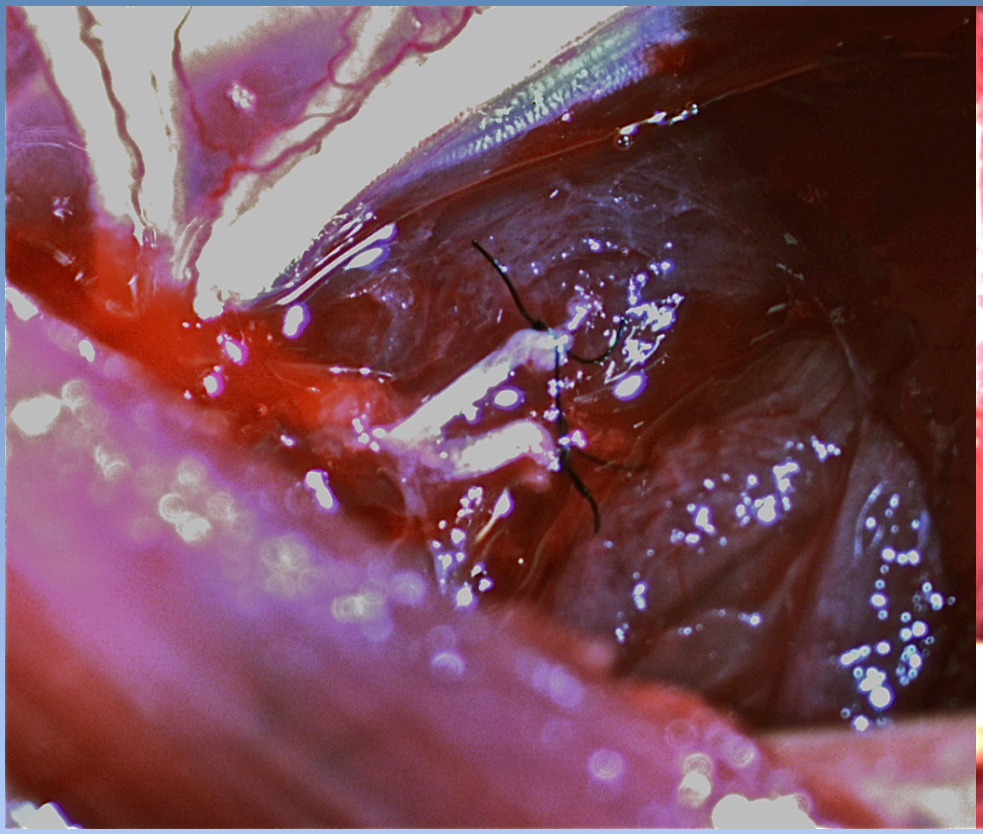


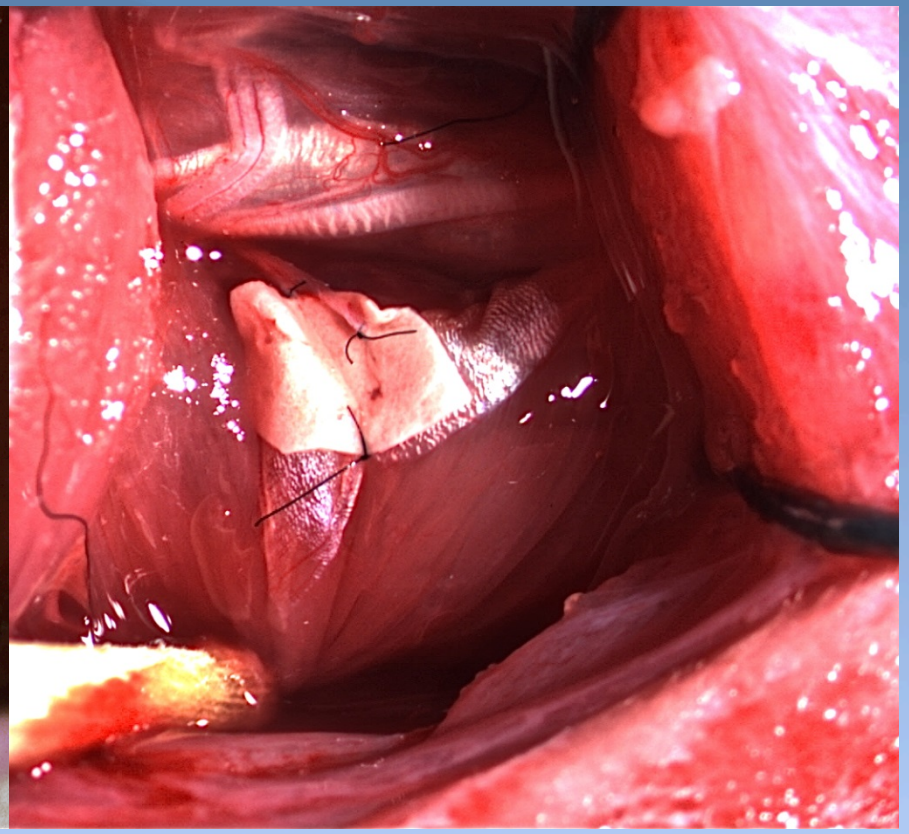
NF Matrix Regeneration Success

Tissue Engineering a Neuromuscular Interface

Tissue Engineering a Neuromuscular Interface







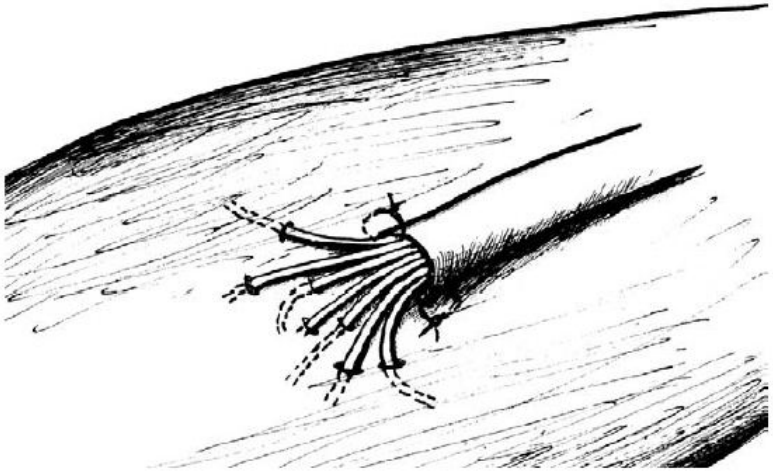


Figure 1. Modified rabbit experiment: the peroneal nerve was divided artificially into 4 or 5 branches implanted as wide as possible to reinnervate the maximum number of muscular fibers.

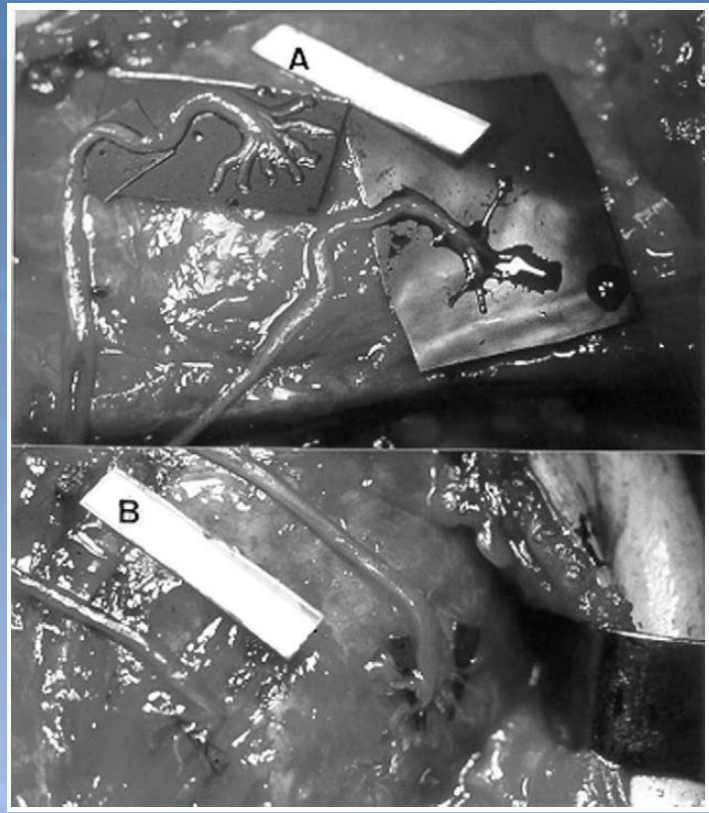
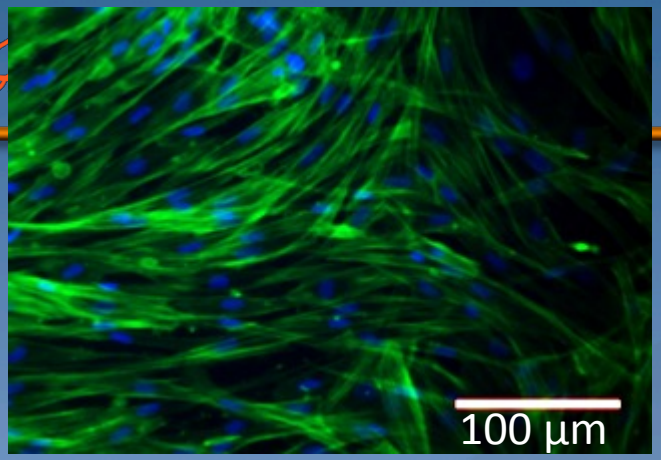
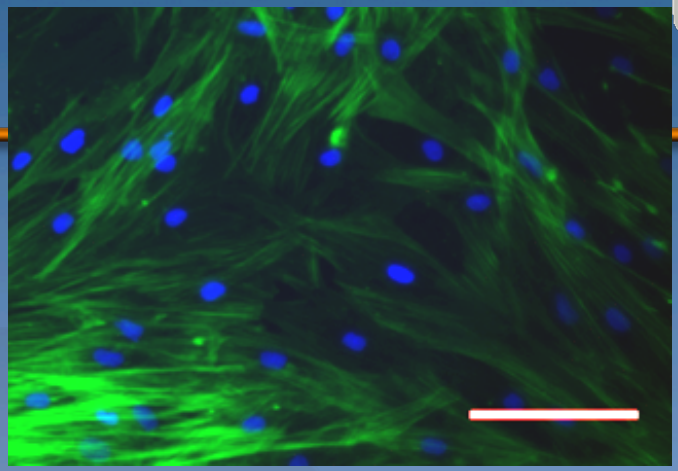


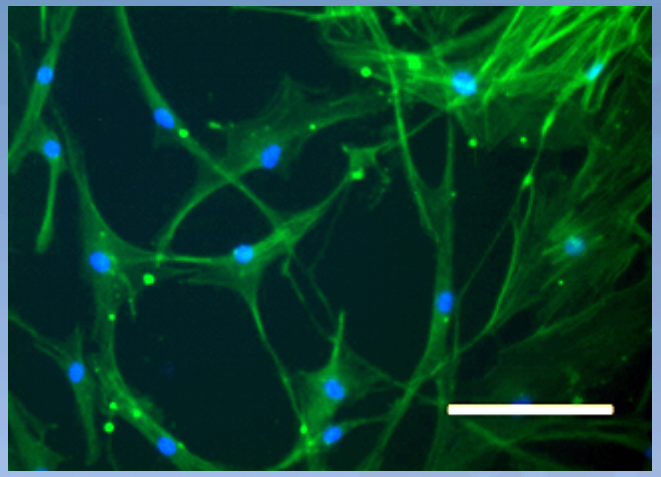
Figure 10. (A) A direct muscular neurotization of the deltoid muscle by means of 2 grafts connected with the proximal stump of the axillary nerve. (B) Same case, the artificial division of the grafts have been introduced into the muscle.



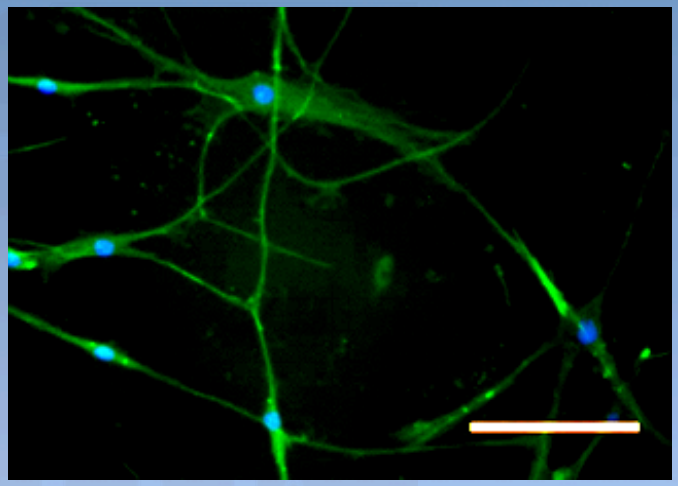
BM-basal media



DM-dorsomorphin BMP pathway inhibitor

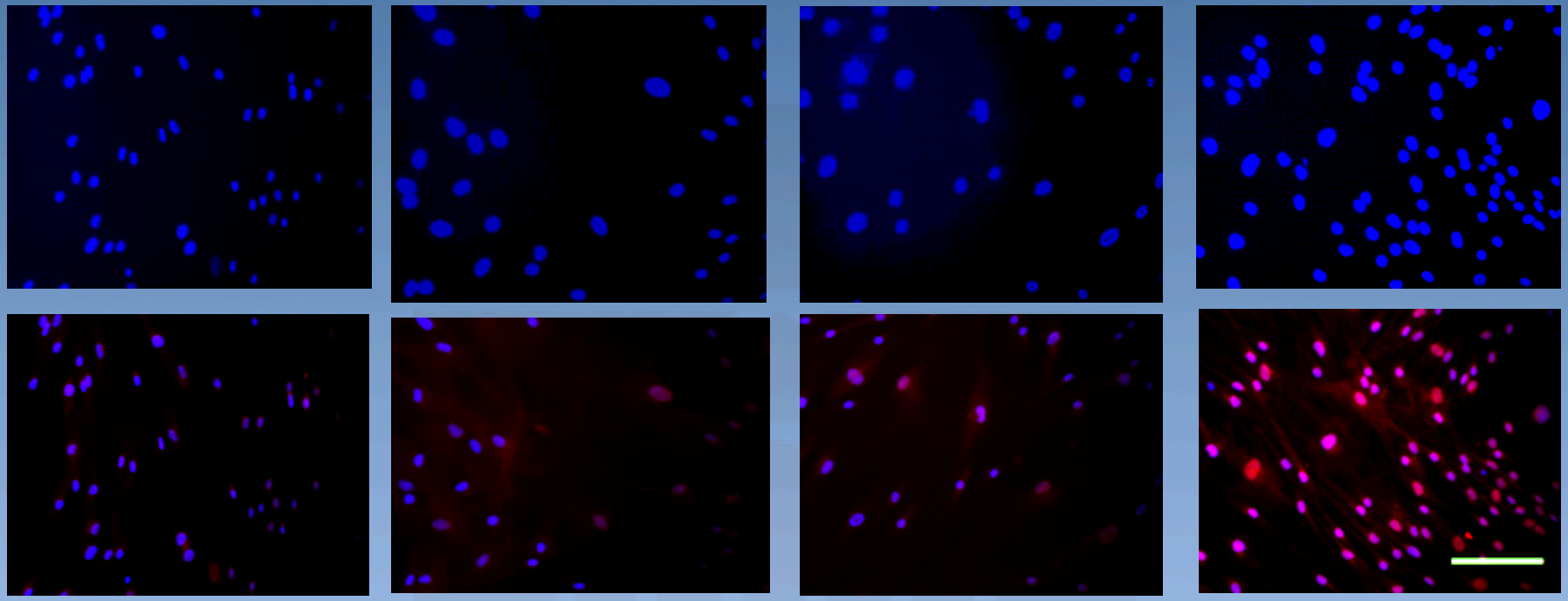


SB-SB431542 TGFβ pathway inhibitor

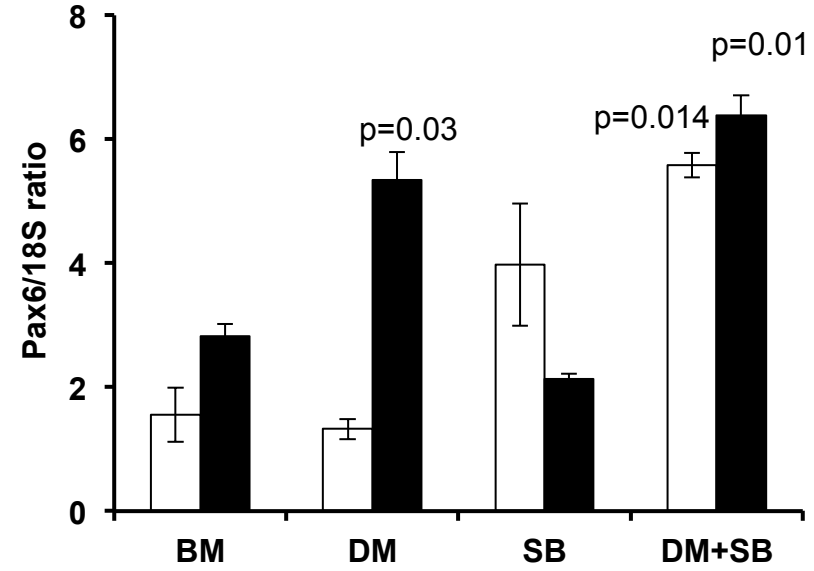


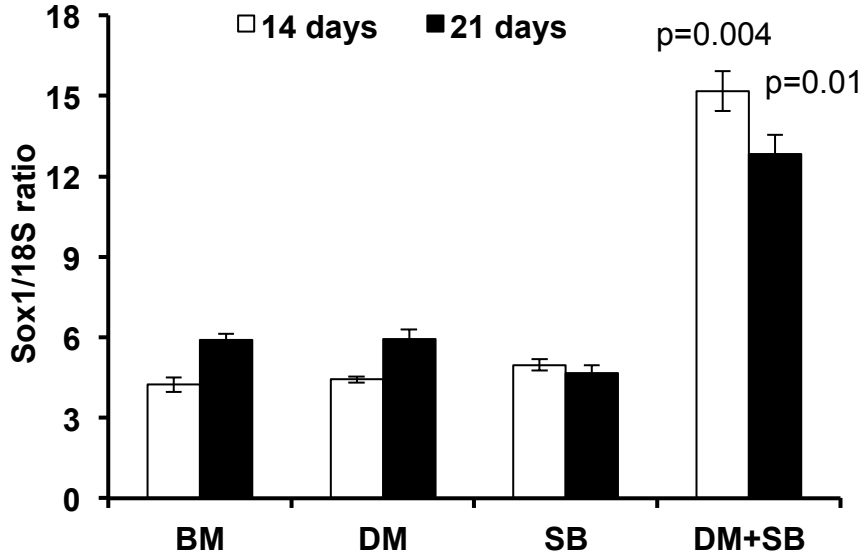
DM+SB

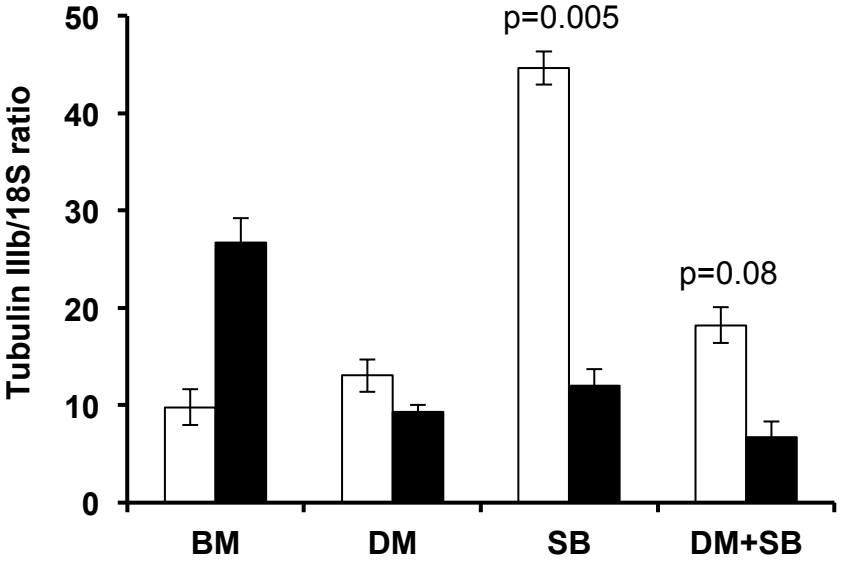
hADSCs express neurite outgrowths with inhibition of TGFβ and BMP pathways- Length of neurite outgrowths significantly longer in DM+SB group

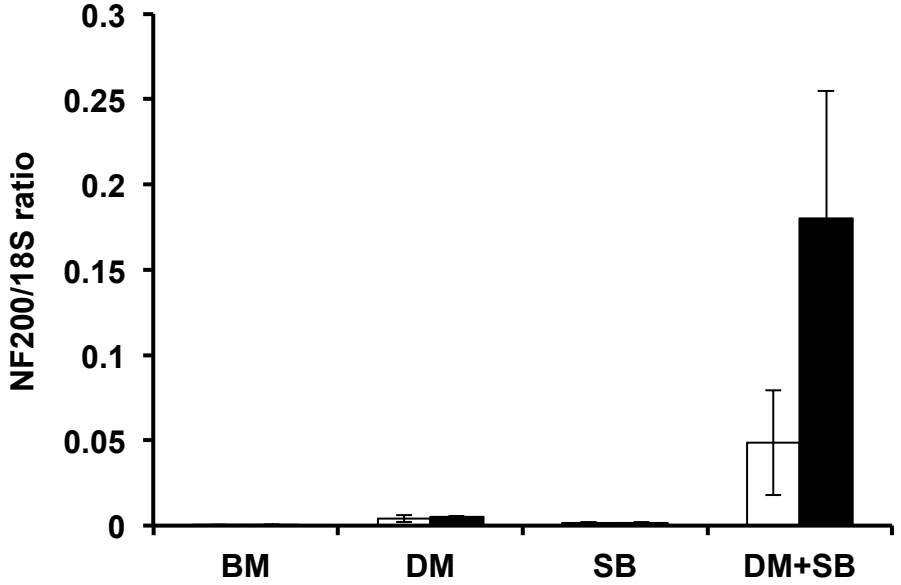


hADSCs express neuron specific enolase upon dual inhibition of TGFβ and BMP inhibition

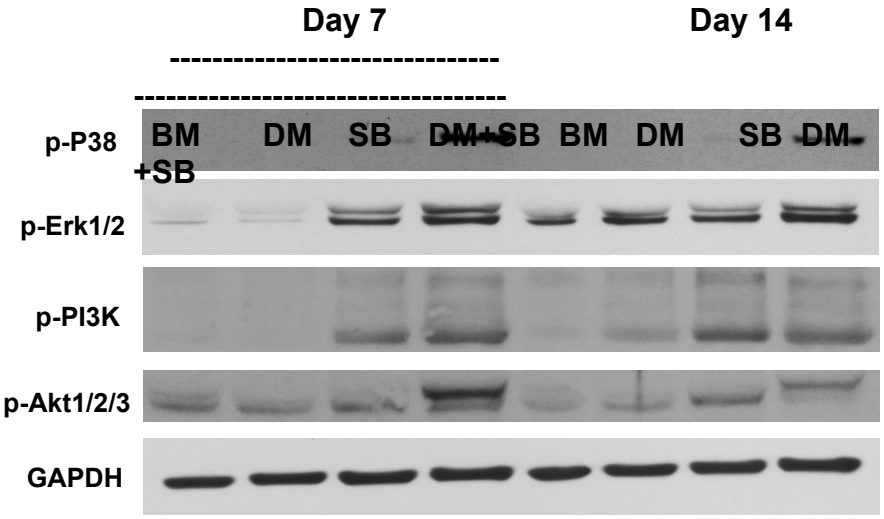


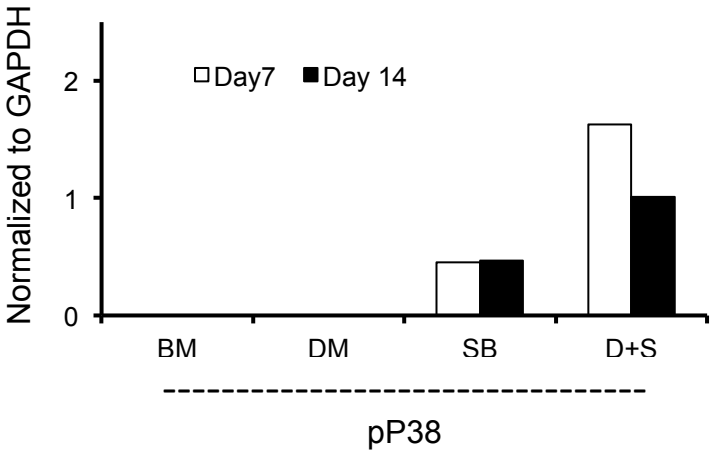


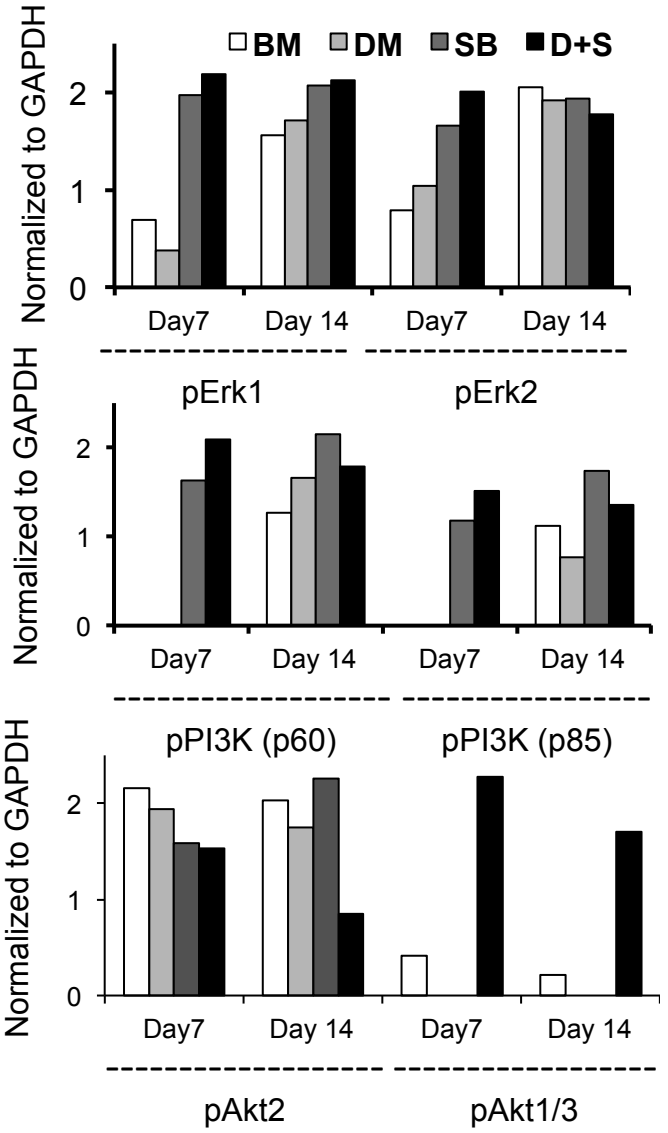


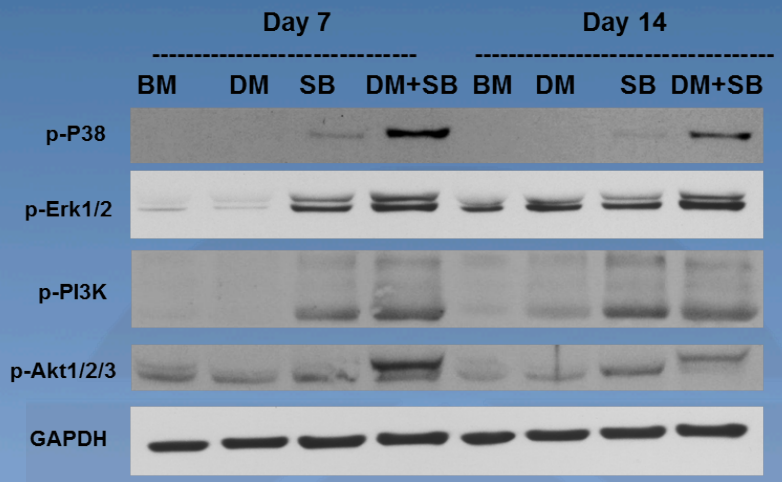


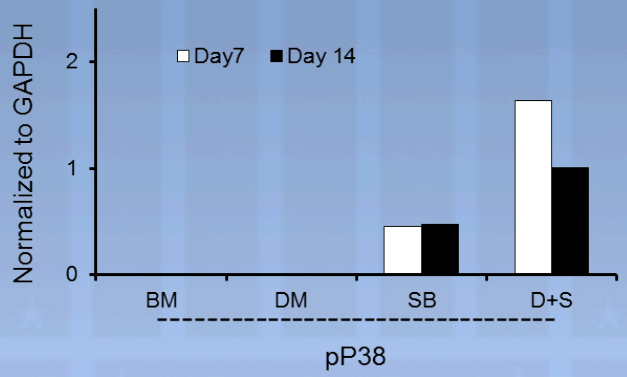
mRNA expression of neuronal markers in hADSCs increases with inhibition of TGFβ and BMP pathways


Day 7
Day 14
p-P38
BM
DM
SB
DM+SB
BM
DM
SB
DM+SB
p-Erk1/2
p-PI3K
p-Akt1/2/3
GAPDH

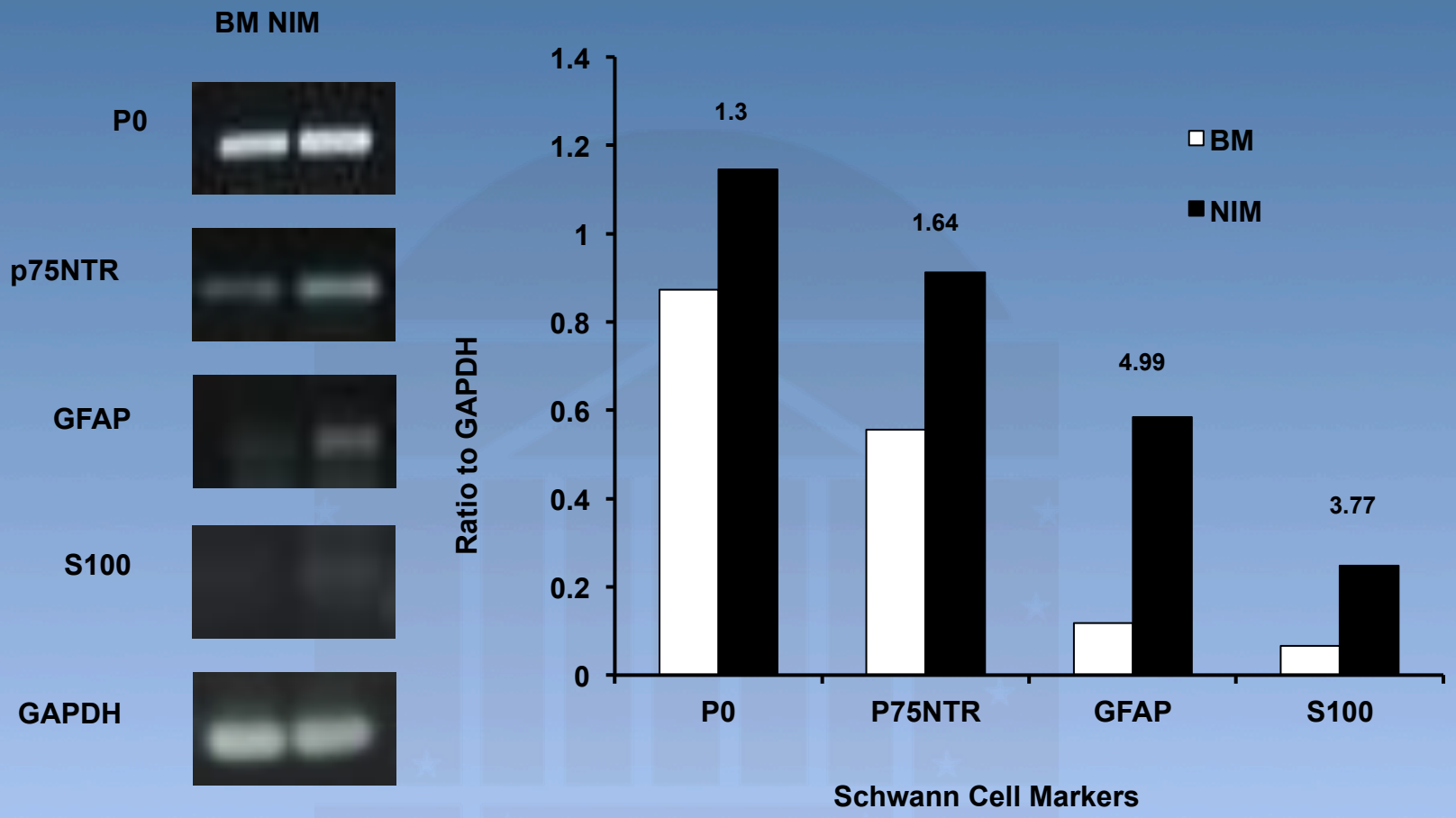

Normalized to GAPDH
Day7
Day 14
0
1
2
BM
DM
SB
D+S
pP38


BM
DM
SB
D+S
Normalized to GAPDH
0
1
2
Day7
Day 14
Day7
Day 14
pErk1
pErk2
pPI3K (p60)
pPI3K (p85)
pAkt2
pAkt1/3

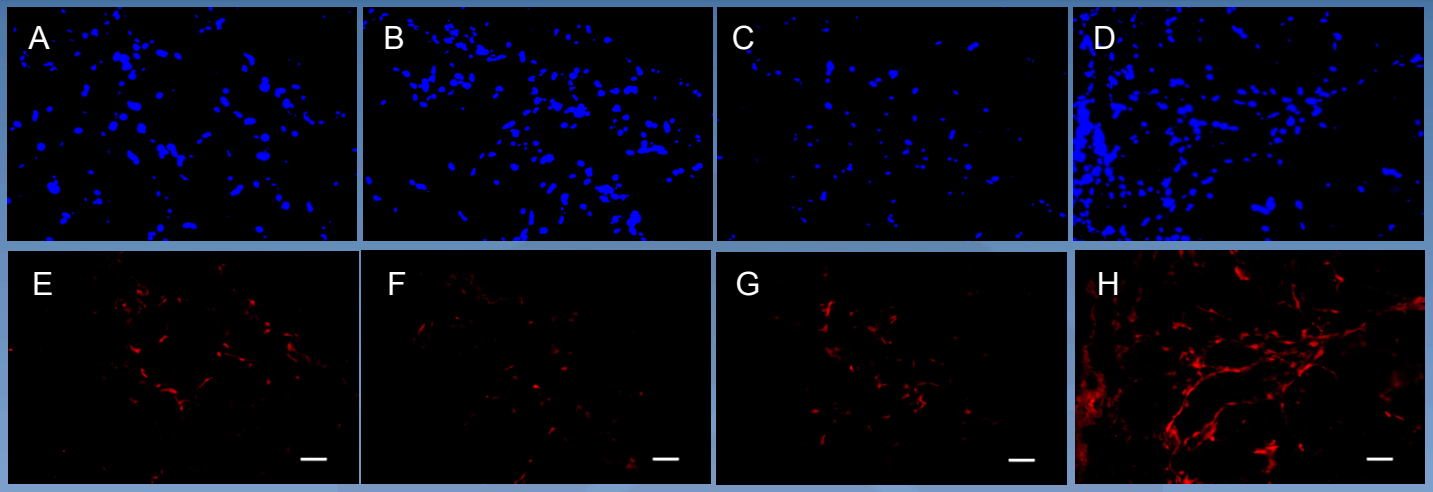


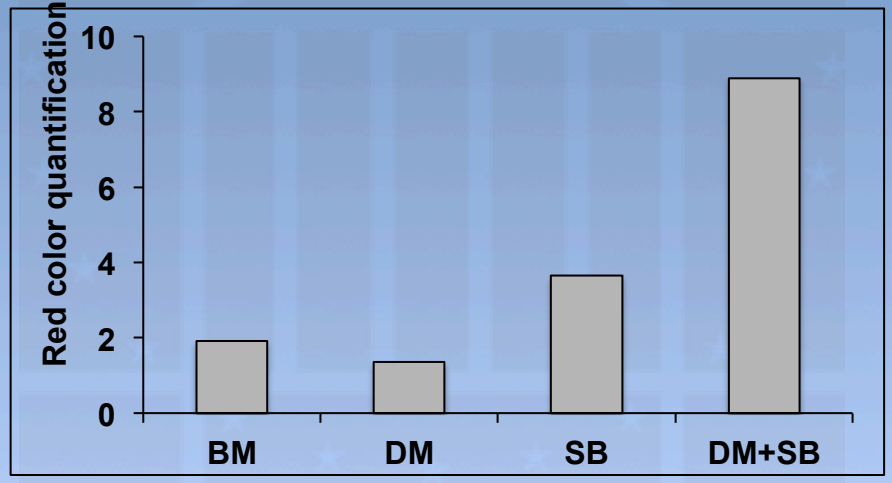


Inhibition of TGFβ and BMP signaling pathways activates p38 in hADSCs. A dynamic balance between activation of p38 and other kinases is known to control neuronal differentiation of stem cells



hADSCs can differentiate into Schwann cell phenotype when treated with NIM





A-D: DAPI/ E-H anti GAP 43 antibody indicating axon growth cone specific GAP 43

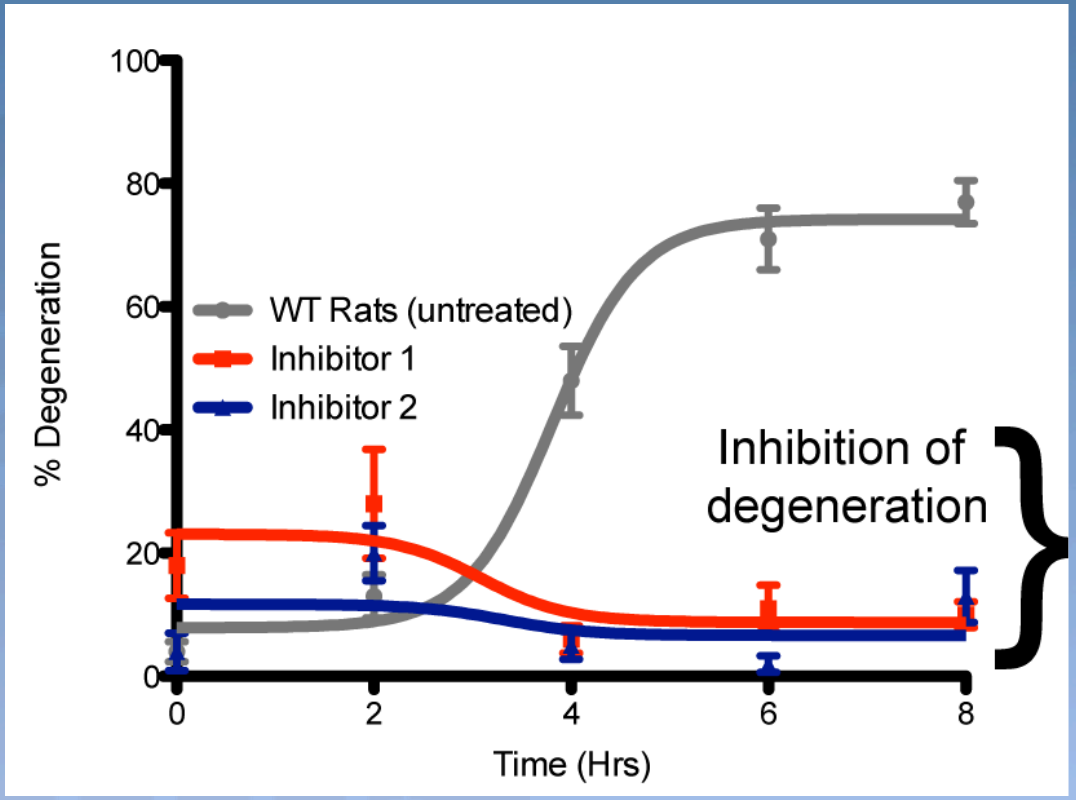
Coulter Grant Application

Overview of milestones:

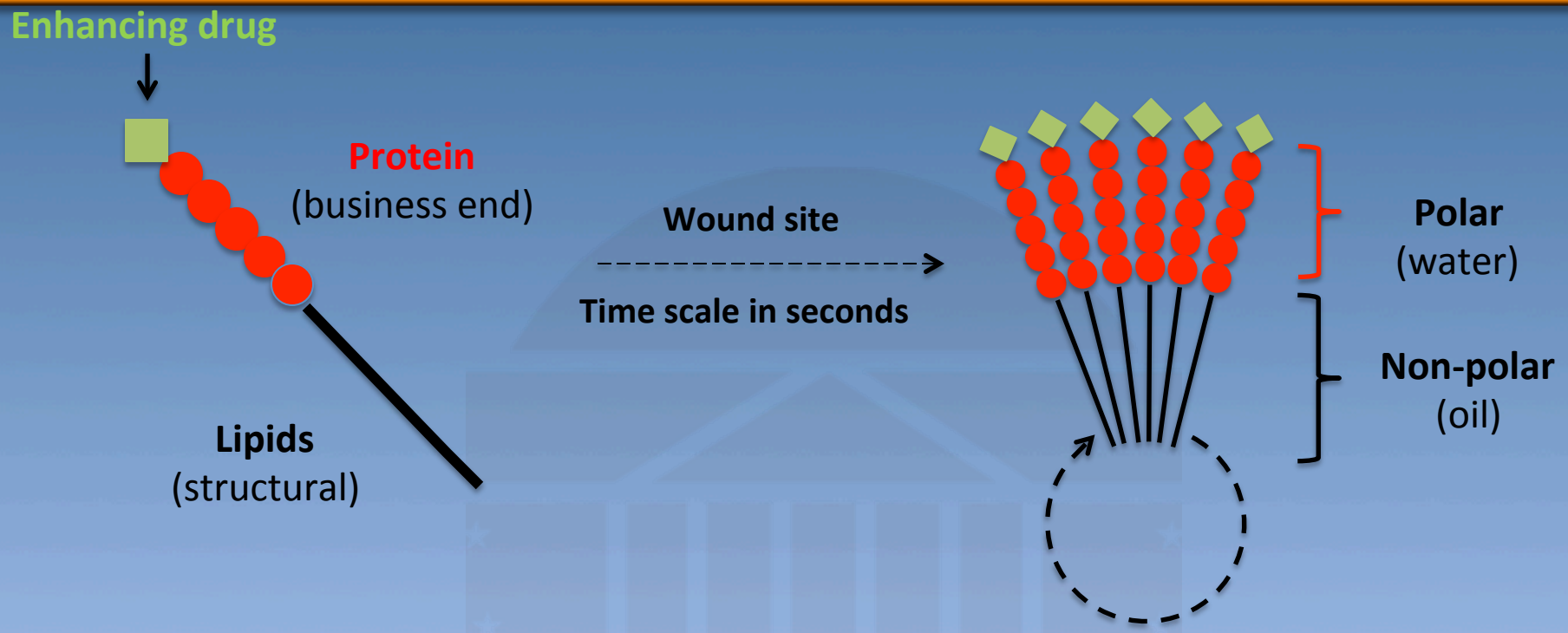
- Milestone 1 (q1): Assess the ability of single compound/nanoscaffold formulations to preventdegeneration, promote regrowth, or support survival of injured neurons.
- Milestone 2 (q2): Assess the ability of combination of the most effective compounds from milestone 1 to prevent degeneration, promote regrowth, **and** support survival of injured neurons.
- Milestone 3 (q3): Test candidate nanoscaffold formulations in a preclinical rat nerve injury model.
- Milestone 4 (q4): Move toward testing promising candidates in a clinical setting.

Coulter Grant Application

- Inhibition of Wallerian degeneration using proprietary compound discovered by Dr. Chris Deppman in the Biology Department

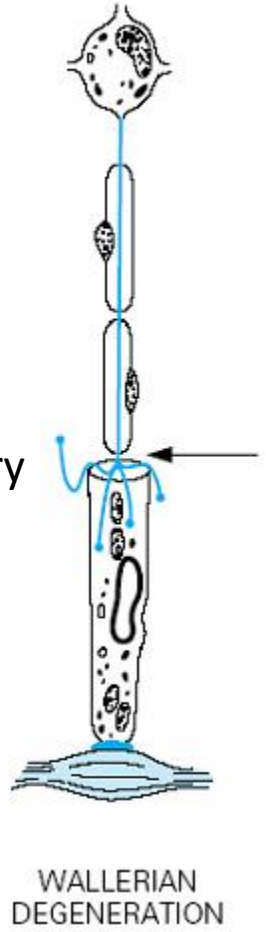


Building the Scaffold



- Simple starting materials (common molecules found in all cells).
- No assembly required! (self-assembly)
- Flexible (multiple) payloads
- Concentration effect

Approach:
Succeeding where others have failed



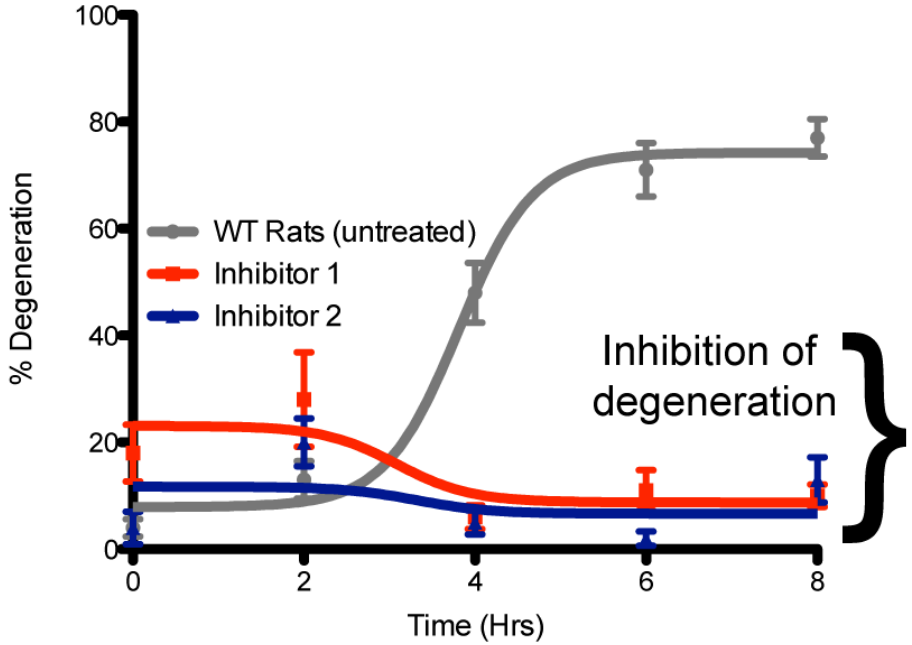
- Path to therapy:

Cell culture→Animal model→Human trials

Rapidly deliverable porous scaffold to cross scarred tissue

Established growth factors and small molecules to promote axon regrowth and maintain cell viability

Proprietary compound(s) that prevent axon degeneration



Coulter Grant

- Rapidly deliverable porous scaffold to cross scarred tissue-self assembling peptide
- Established growth factors and small molecules to promote axon regrowth and maintain cell viability
- Proprietary compound(s) that prevent axon degeneration

Coulter Grant Application

•Biocompatible – minimal immune response; biodegradable

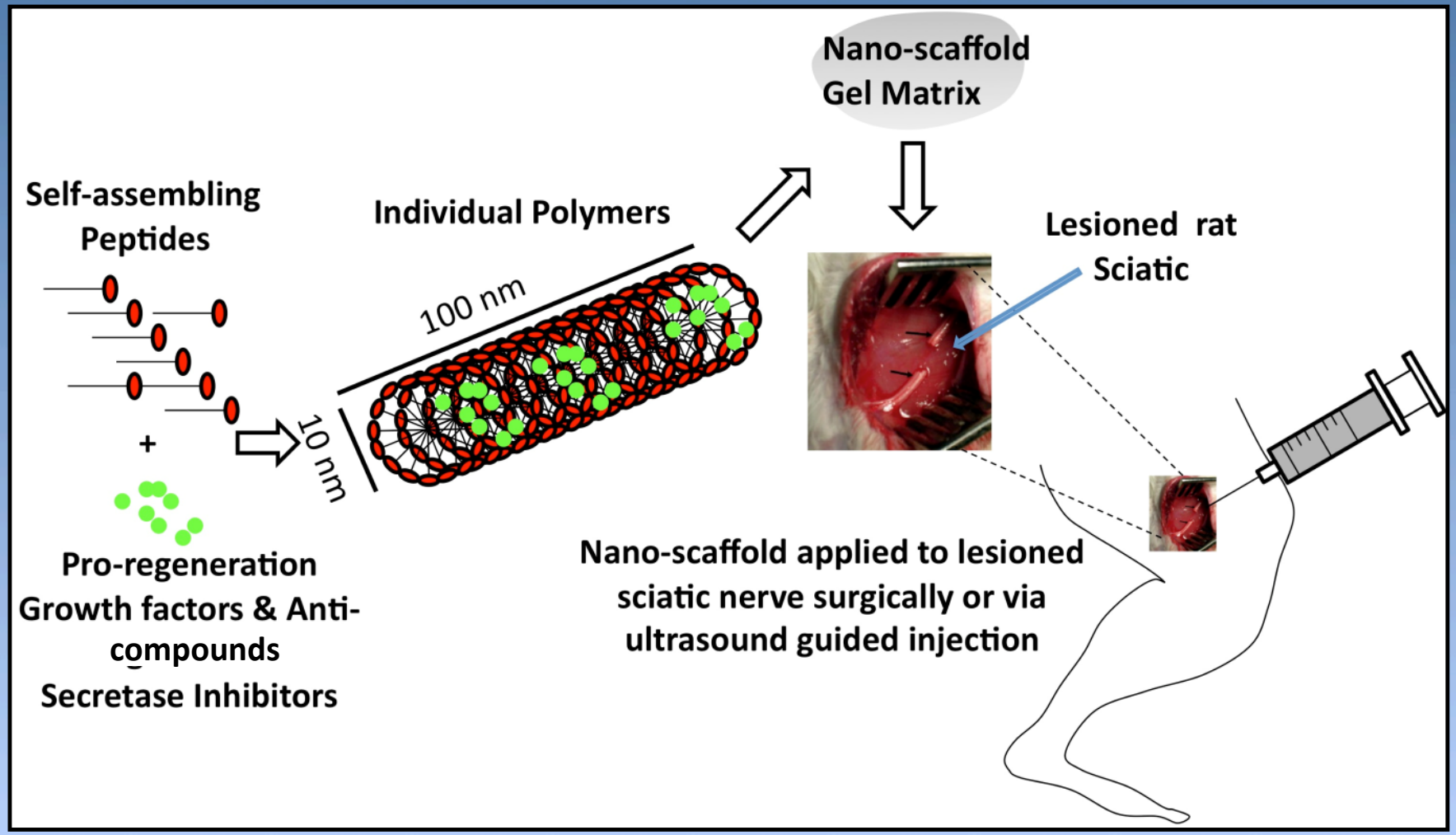
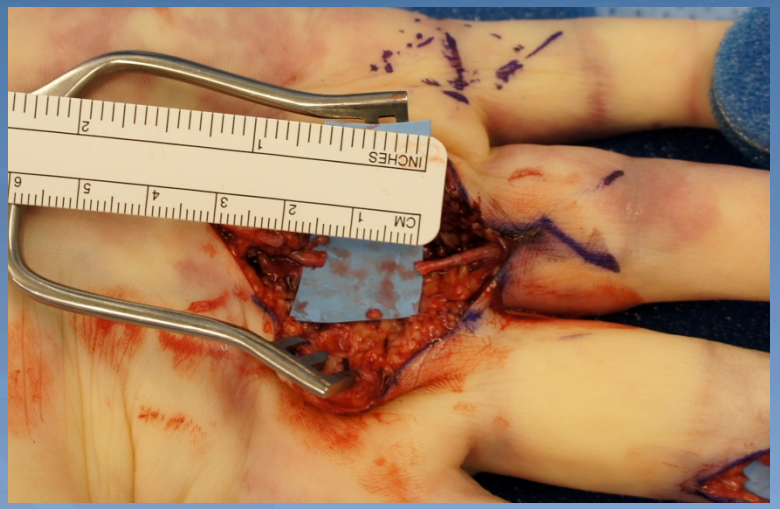
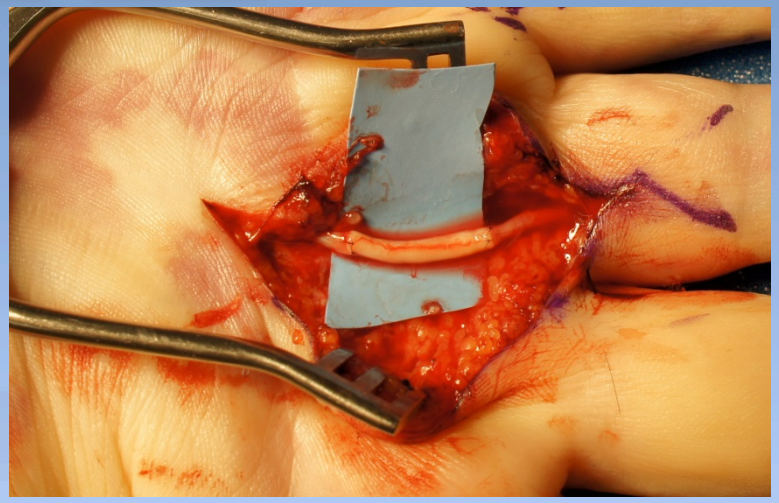


Figure 2: Schematic of Preclinical Strategy for Nerve Repair

Axogen Trial

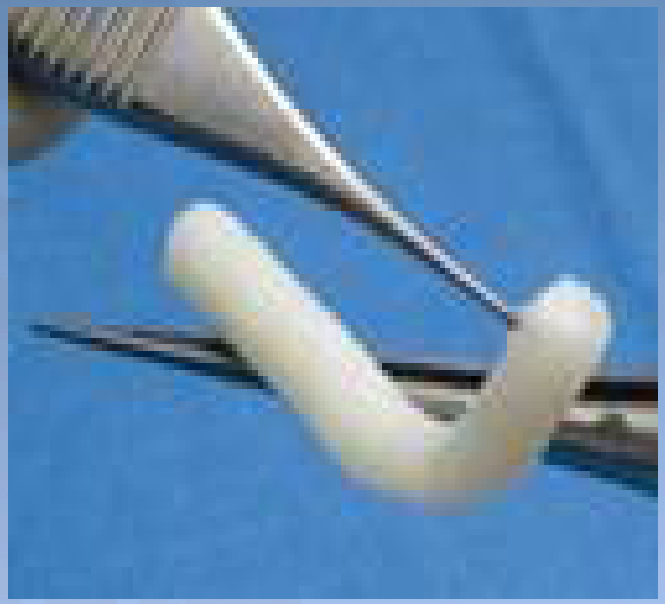
- Allograft digital nerves
- 15 Centers
- 10 patients per site
- Common or proper digital nerves
- 15-30 mm defects
- Beginning in July (hopefully)…





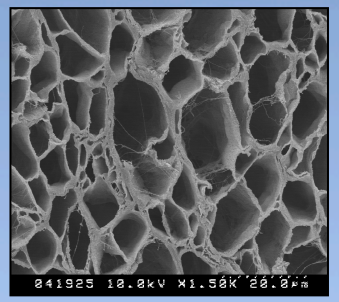
Acellular Allograft

- Abundant supply, no donor site morbidity, decreased surgical time
- Processing advances
  - Removal of debris (chondroitin sulfate proteoglycans)
  - Remove Schwann cells
  - Preserve architecture
  - Immunotolerant



Acellular Allograft

- Animal data clearly better than conduits
  - Almost as good as autograft
  - Up to 3-5cm (87% M3 or better)
  - Schwann cell migration is length limiting obstacle
  - Human controlled clinical trial data needed (multicenter prospective trial comparing allograft vs collagen conduit beginning soon)



Acellular Allograft

Rat Model comparison of autograft, allograft and collagen conduit

At 12 weeks, the mean muscle force as compared with that on the contralateral (control) side:

- autograft group 45.2% ± 15.0%
- allograft group 43.4% ± 18.0%
- collagen group 7.0% ± 9.2%

Giusti G, Willems WF, Kremer T, Friedrich PF, Bishop AT, Shin AY. Return of motor function after segmental nerve loss in a rat model: comparison of autogenous nerve graft, collagen conduit, and processed allograft (AxoGen). J. Bone Joint Surg. Am., 2012; 94: 410-7.

Acellular Allograft

Retrospective Chart Review of the RANGER database (allograft database) upper extremity nerve grafts 56 subjects/ 71 nerves

Meaningful recovery (S3/M3) was reported in:

- 31 of 35 digital nerve repairs (89%)
- 6 of 8 median nerve repairs (75%),
- 2 of 3 ulnar nerve repairs (67%)
- For 100% of all nerve gaps under 15mm

Cho MS, Rinker BD, Weber RV, Chao JD, Ingari JV, Brooks D, Buncke GM. Functional outcome following nerve repair in the upper extremity using processed nerve allograft. J. Hand Surg. Am., 2012; 37: 2340-9.

Acellular Allograft

- 14 patients with 18 digital nerve lacerations
- Defect averaged 11mm (5-30mm)
- Graded using the Taras scale (incorporates static and moving 2 pt discrimination)
  - Excellent Results: 7 (39%)
  - Good Results: 8 (44%)
  - Fair Results: 3 (17%)
  - Poor results: 0
- Small sample size but promising

J Hand Surg Am. 2013 Oct;38(10):1965-71.**Allograft reconstruction for digital nerve loss.**
Taras JS[1], Amin N, Patel N, McCabe LA.

Questions