Preventing Degeneration of the Neuromuscular Junction

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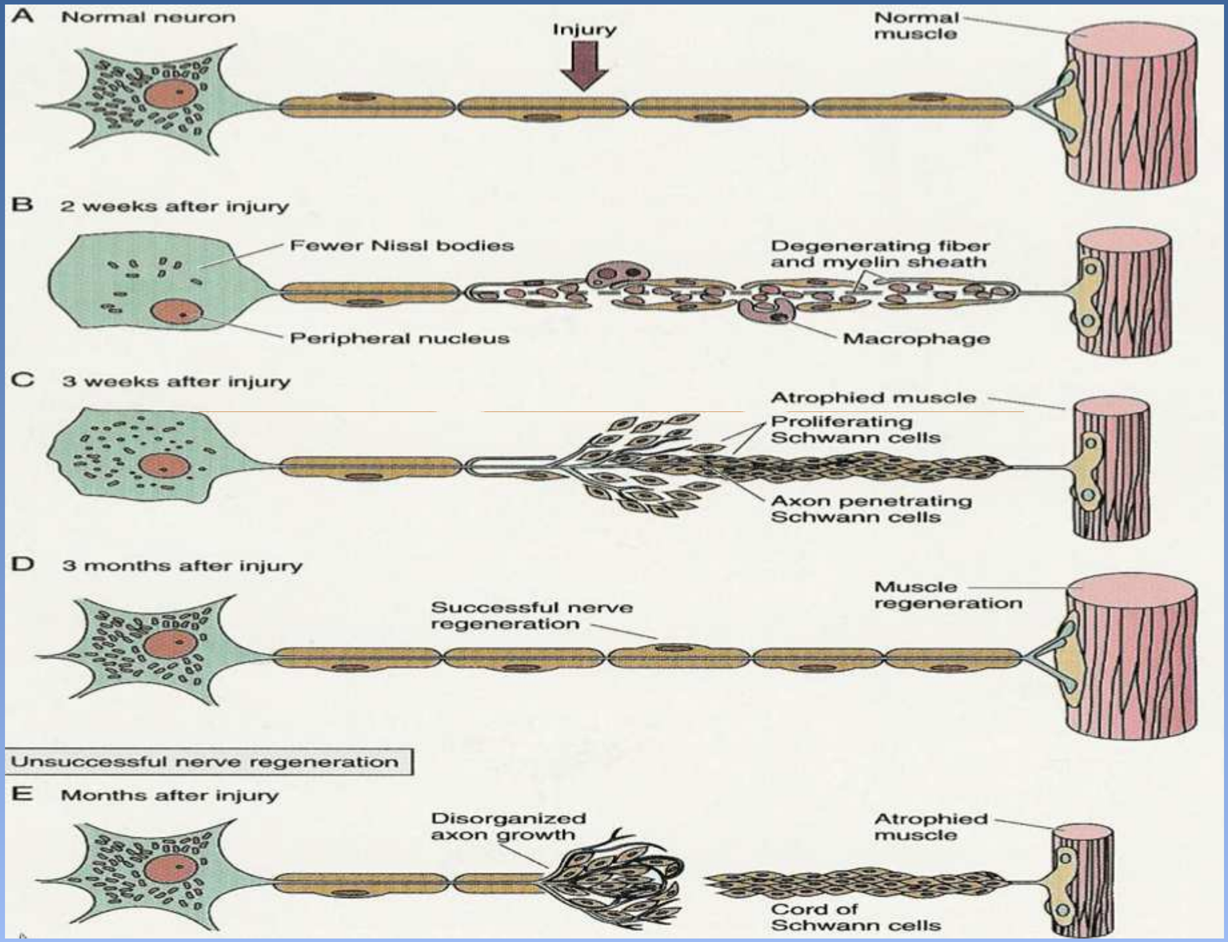
Nerve Injury



The Neuromuscular Junction

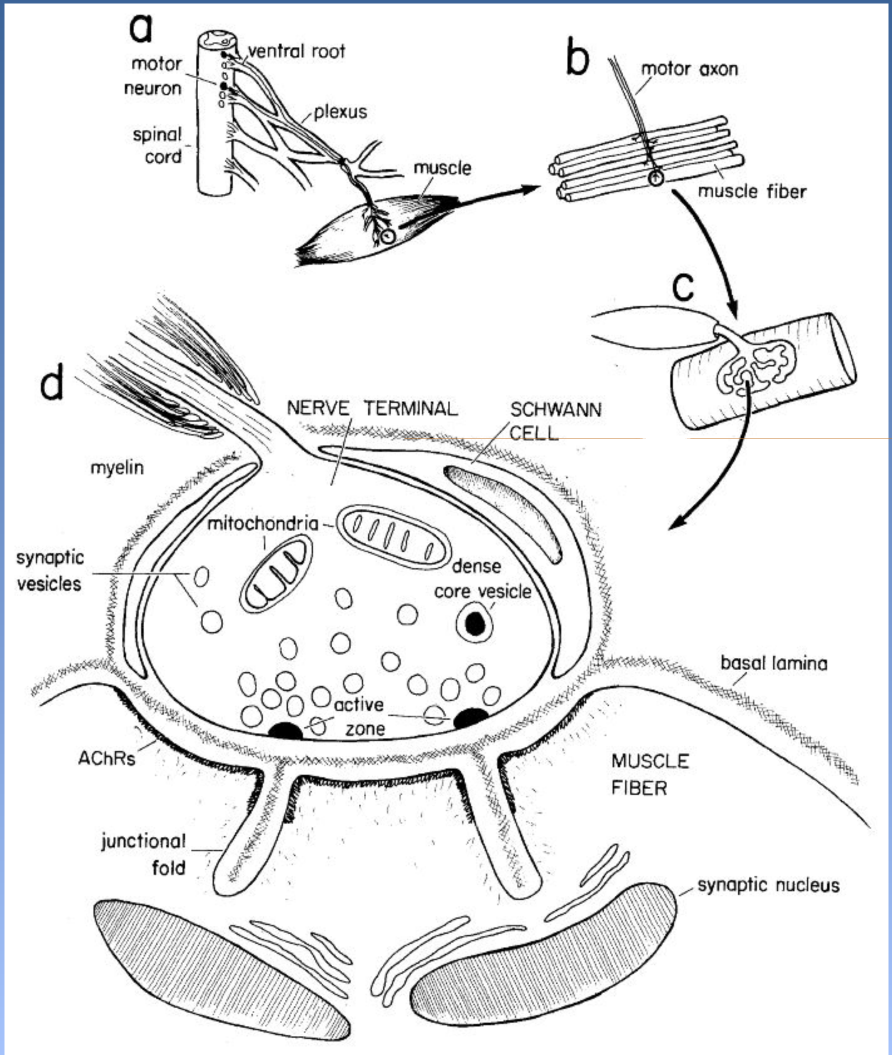
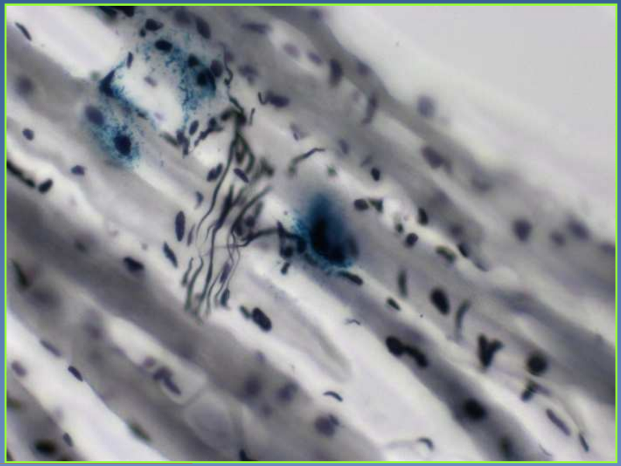
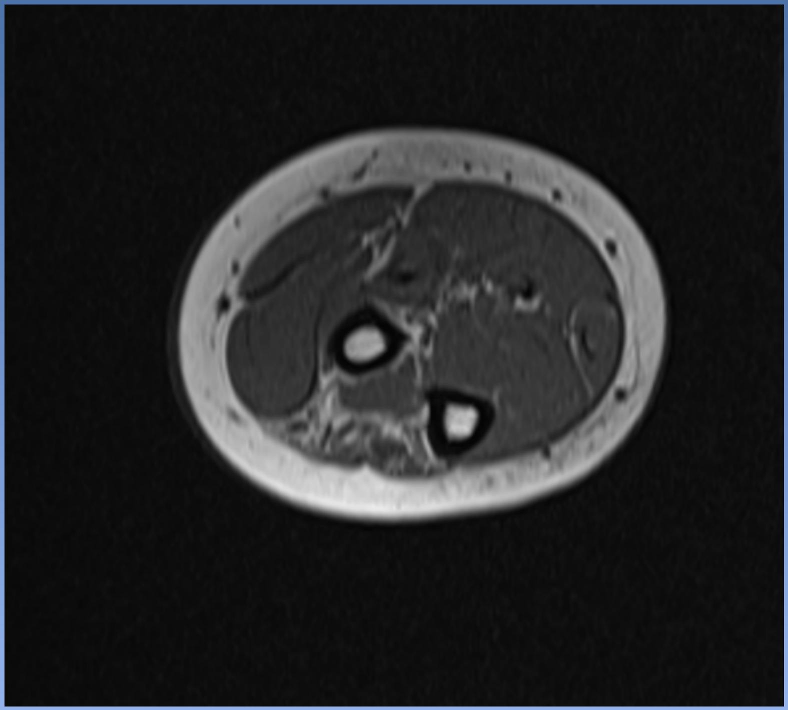


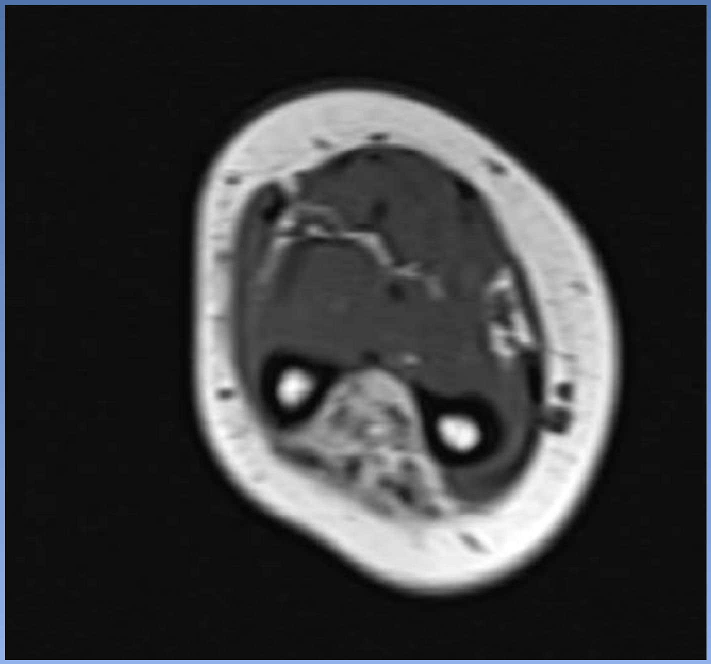
Figure 2. The Adult Neuromuscular Junction



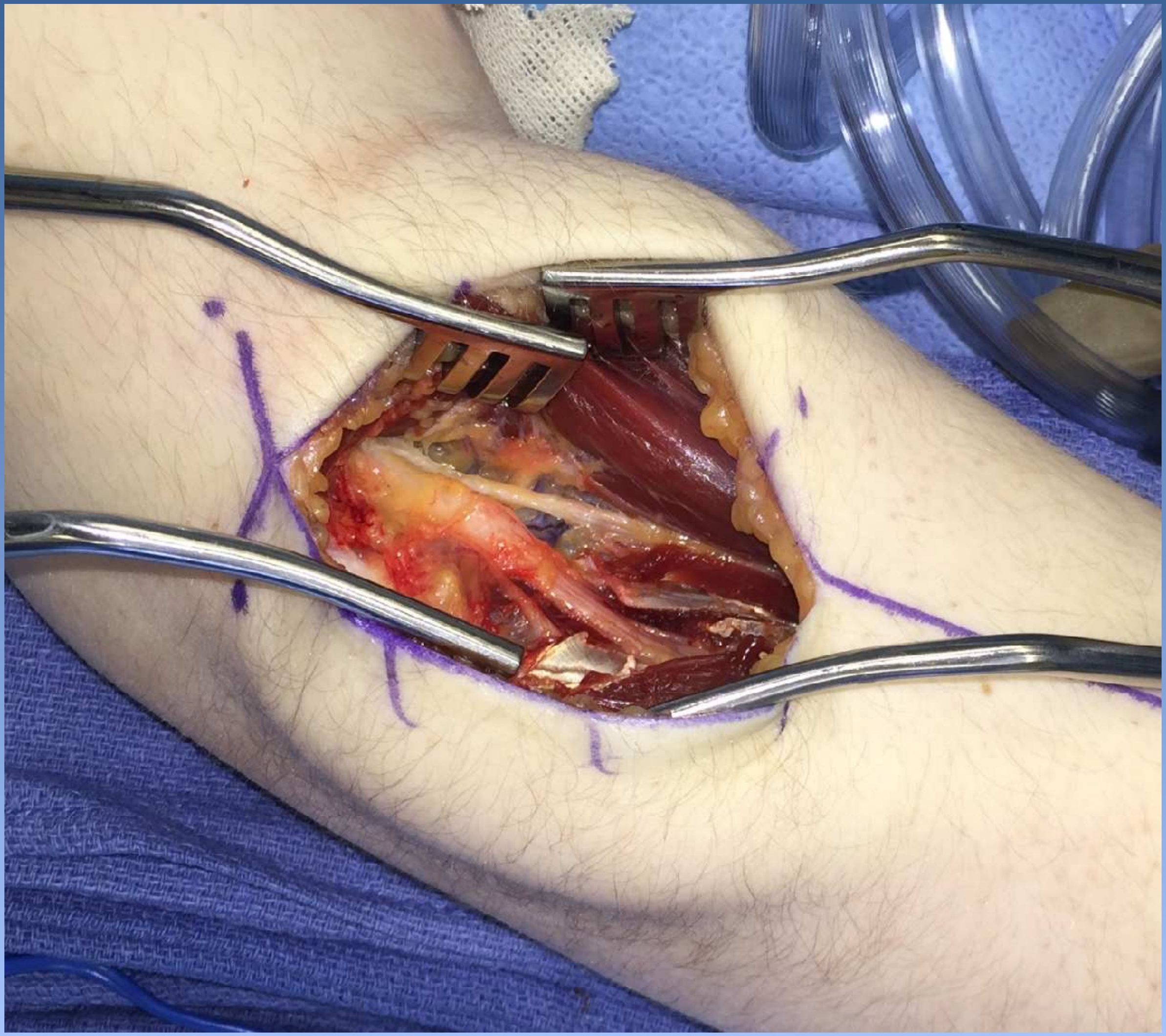
The Clinical Problem

The Clinical Problem

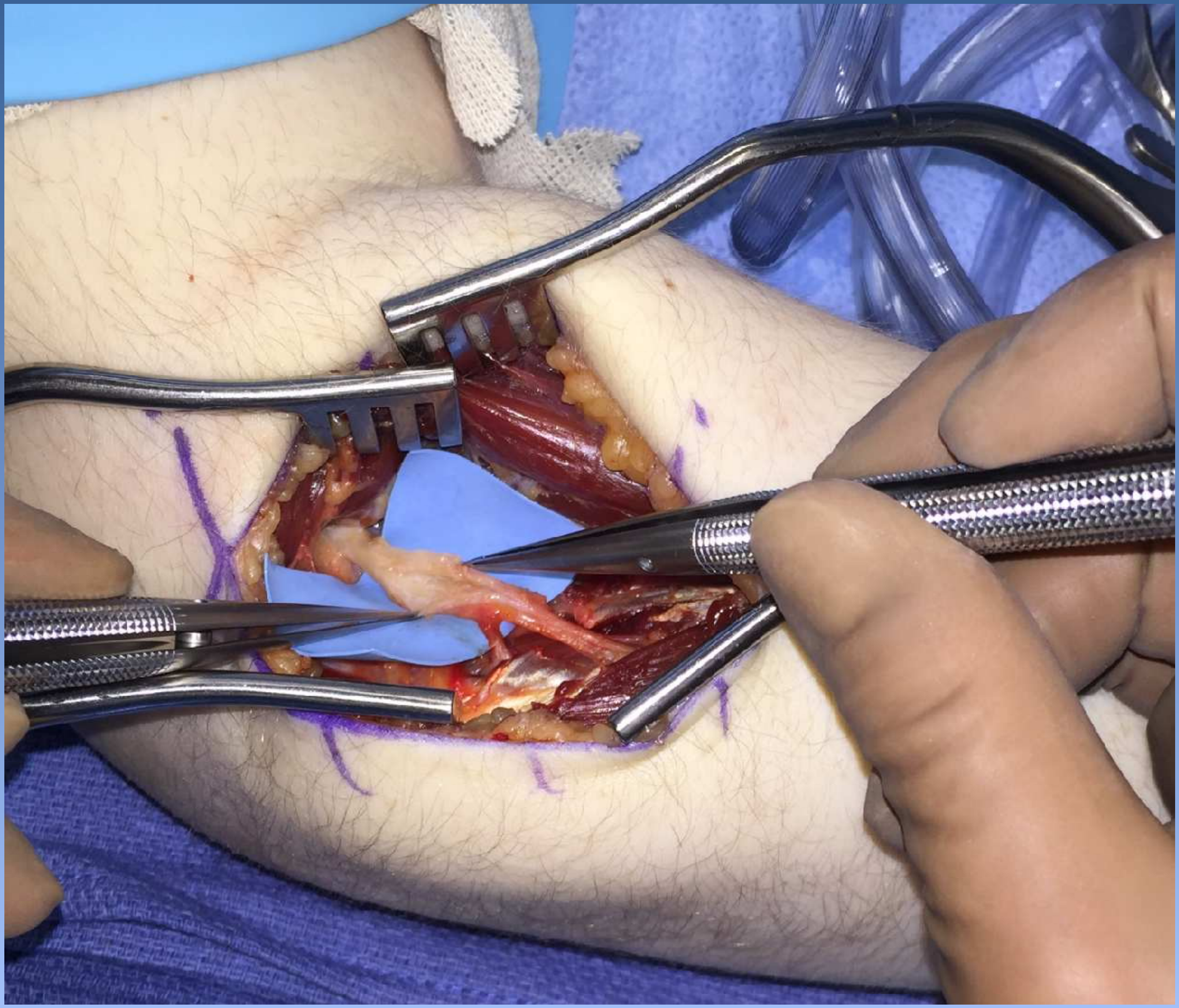




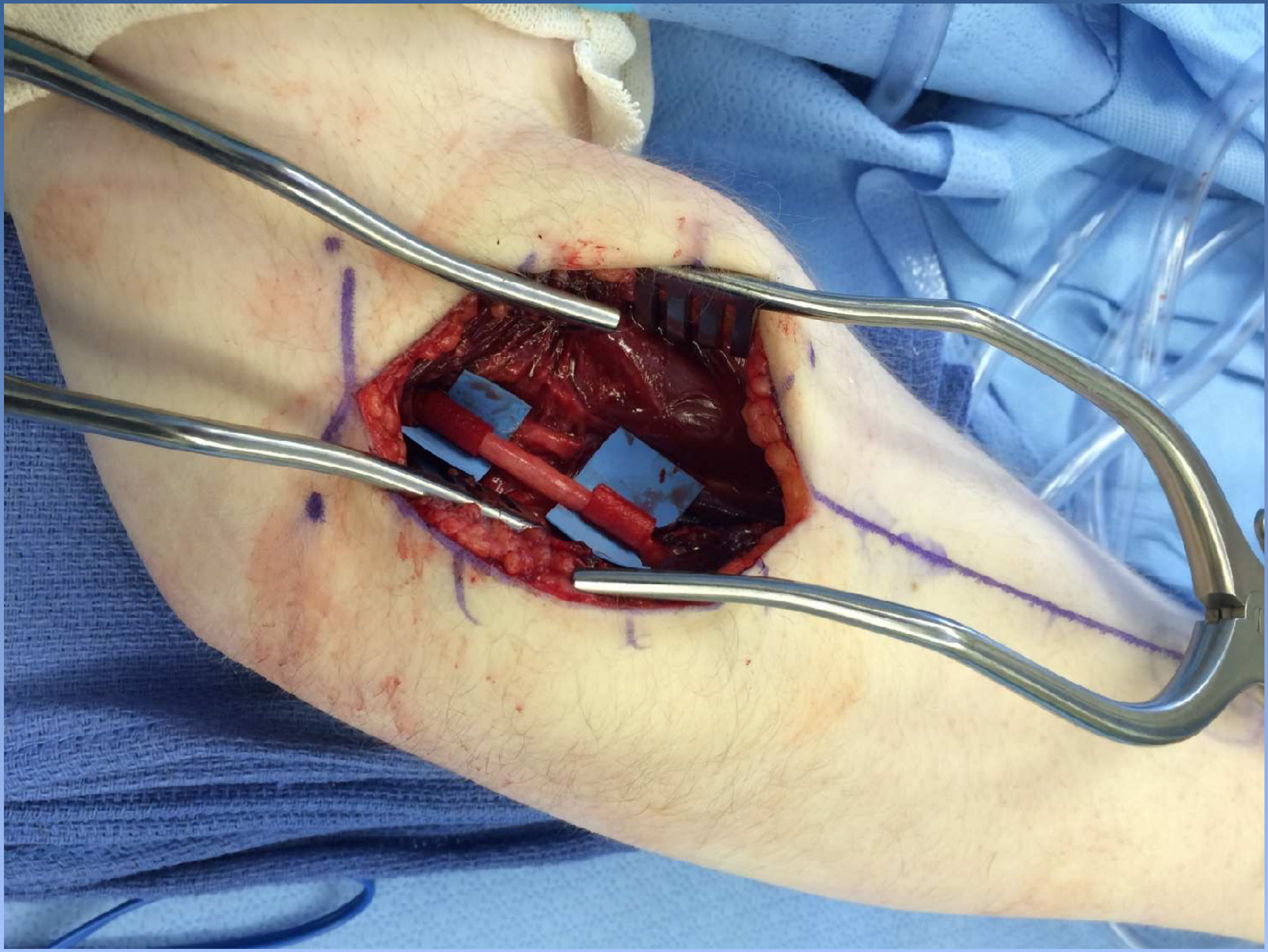
The Clinical Problem



The Clinical Problem

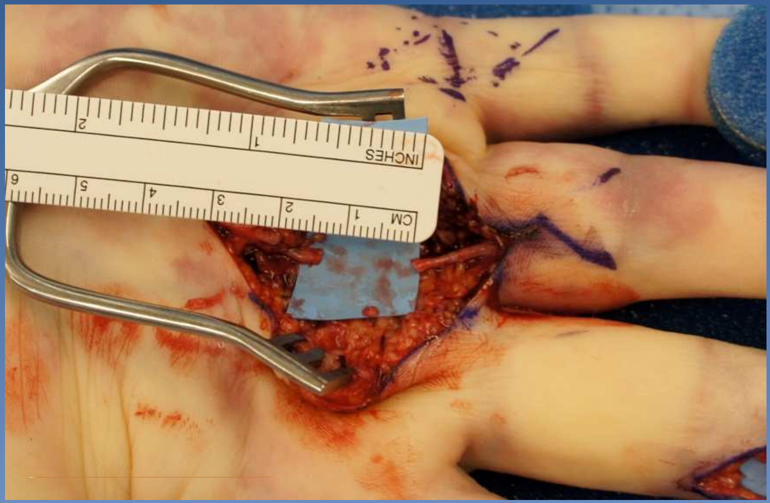


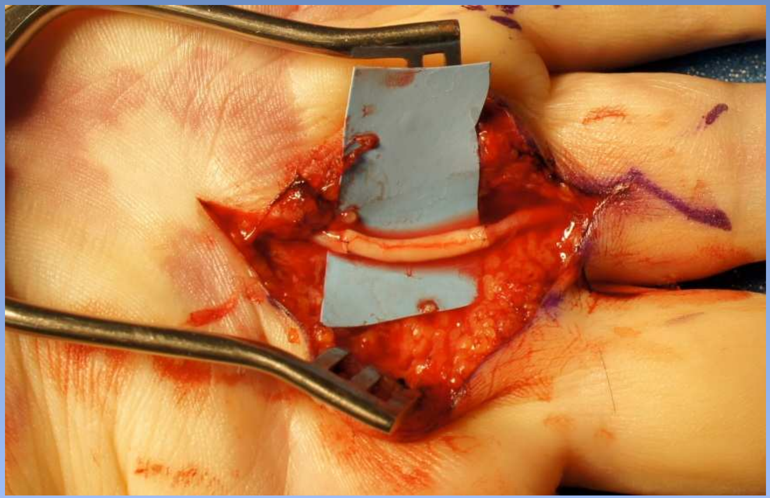
The Clinical Problem



Axogen Trial

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



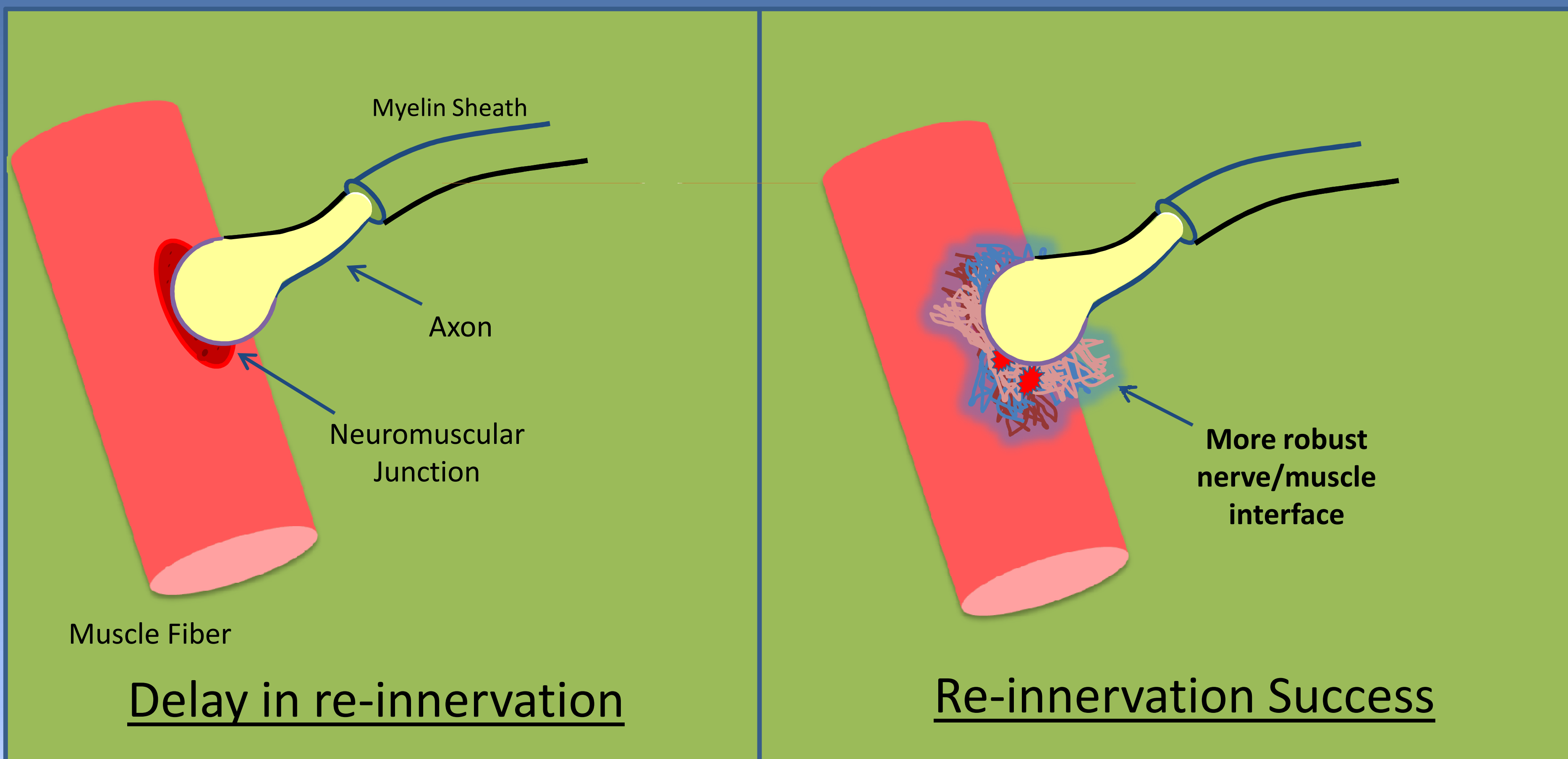


Axogen Trial

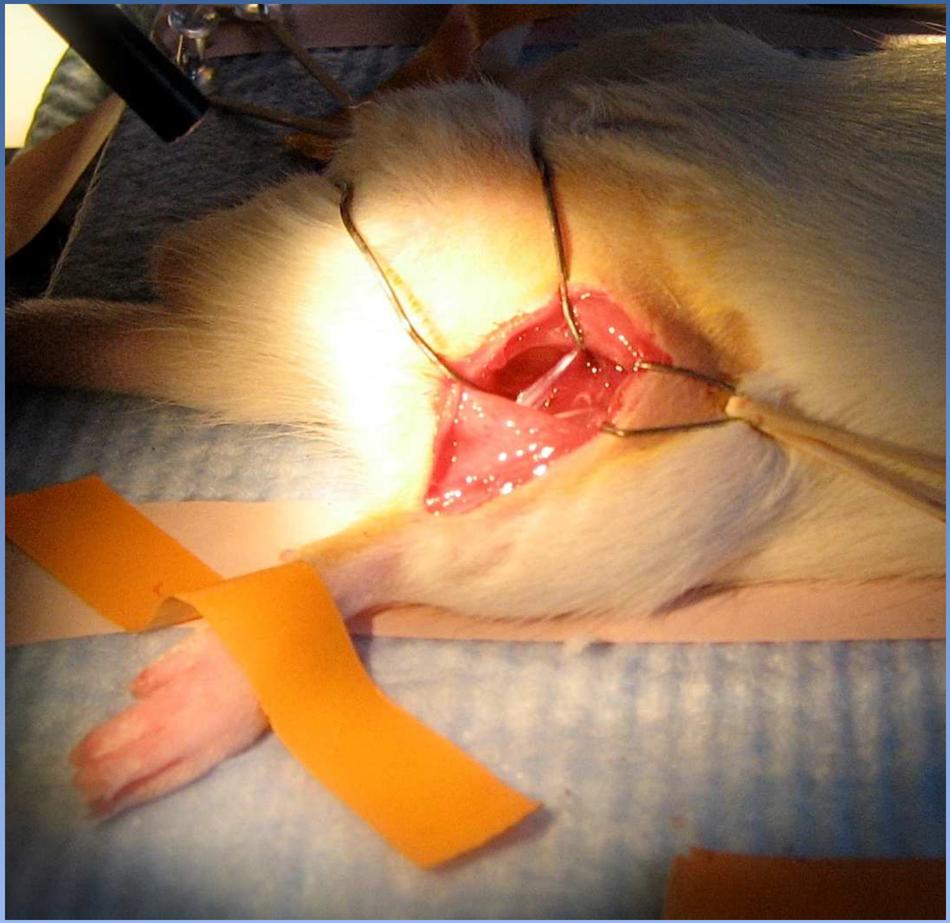
- Acellular allograft abundant supply, no donor site morbidity, decreased surgical time
- Currently looking at digital nerves compared to conduits
- UVA Hand Division 1 of 15 sites in US

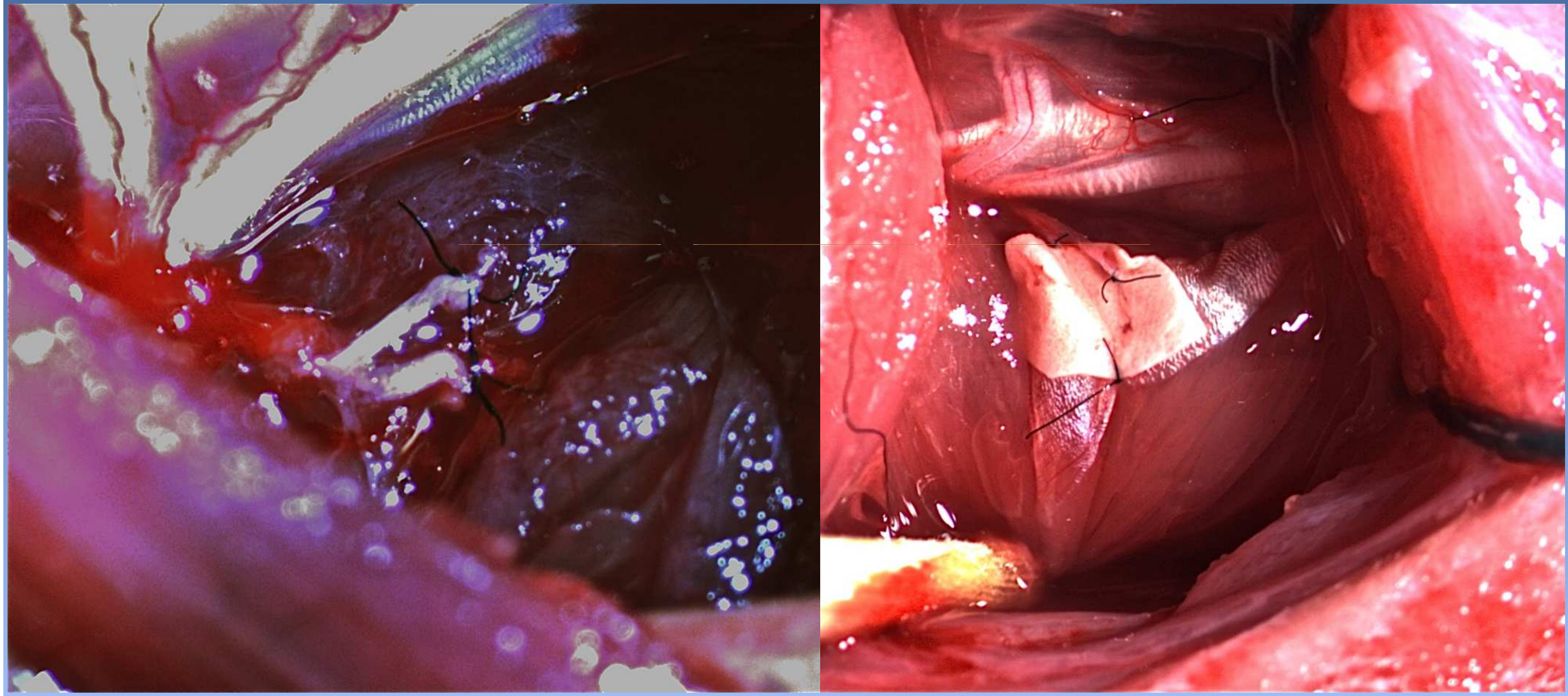
Objective

- Goal: Develop a way to prevent involution of the neuromuscular junction while nerve recovery occurs



Tissue Engineering a Neuromuscular Interface





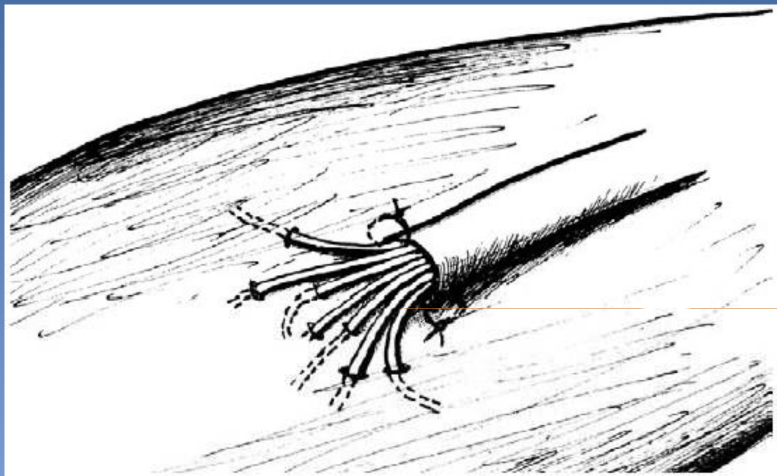
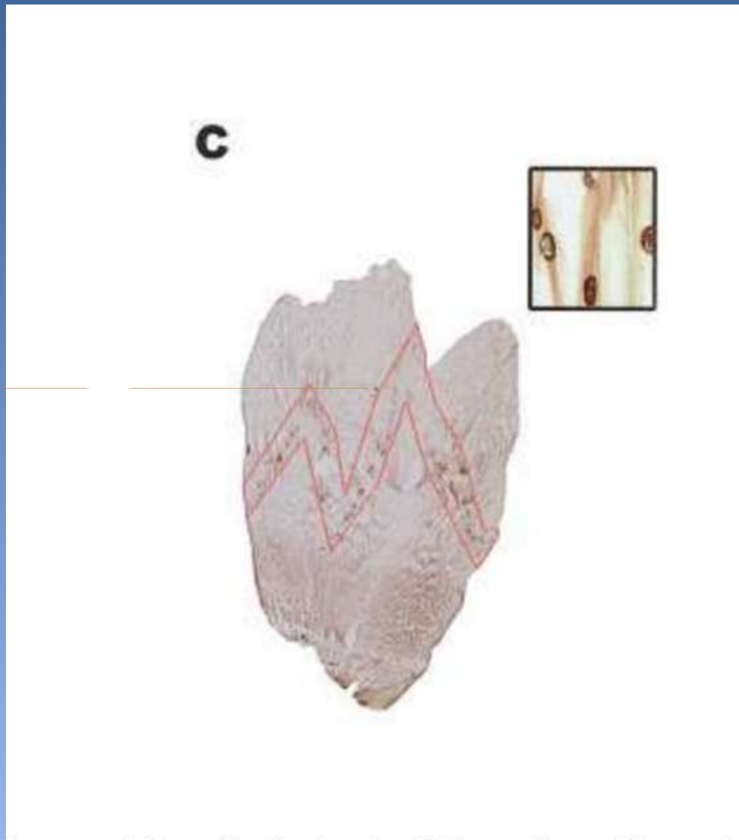
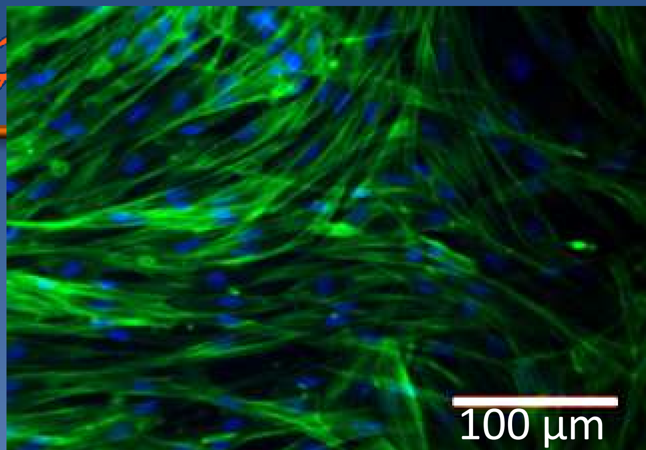
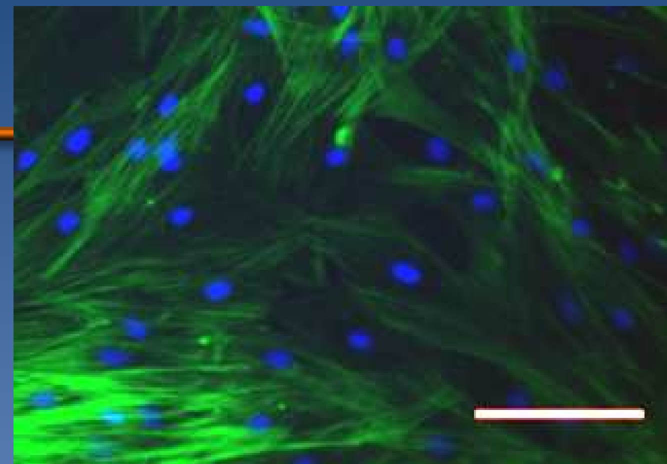


Figure 1. Modified rabbit experiment: the peroneal nerve was divided artificially into 4 or 5 branches implanted as wide as possible to reinnervate the maximum number of muscular fibers.

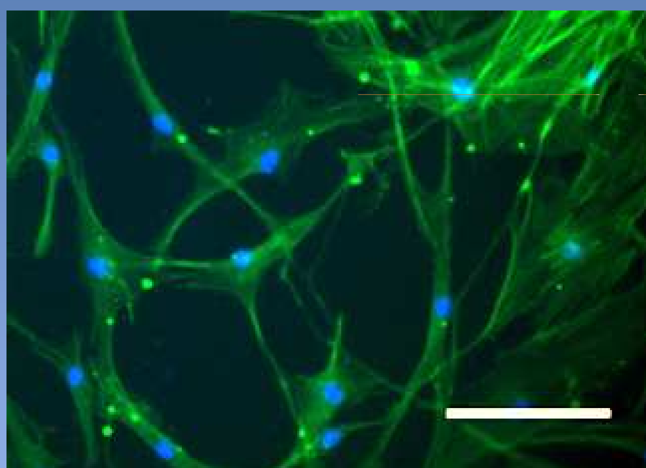




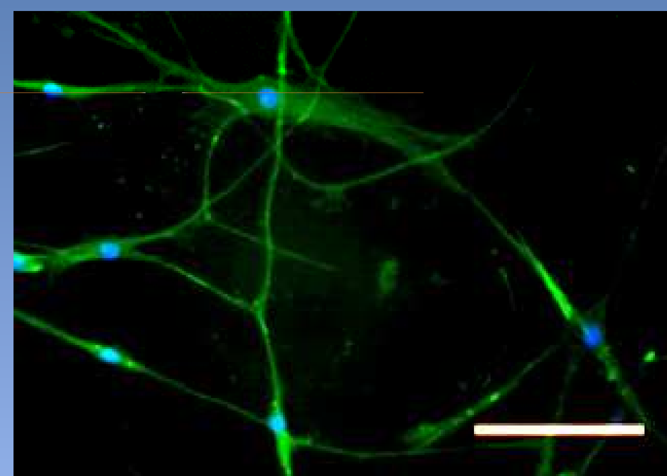
BM-basal media



DM-dorsomorphin BMP pathway inhibitor

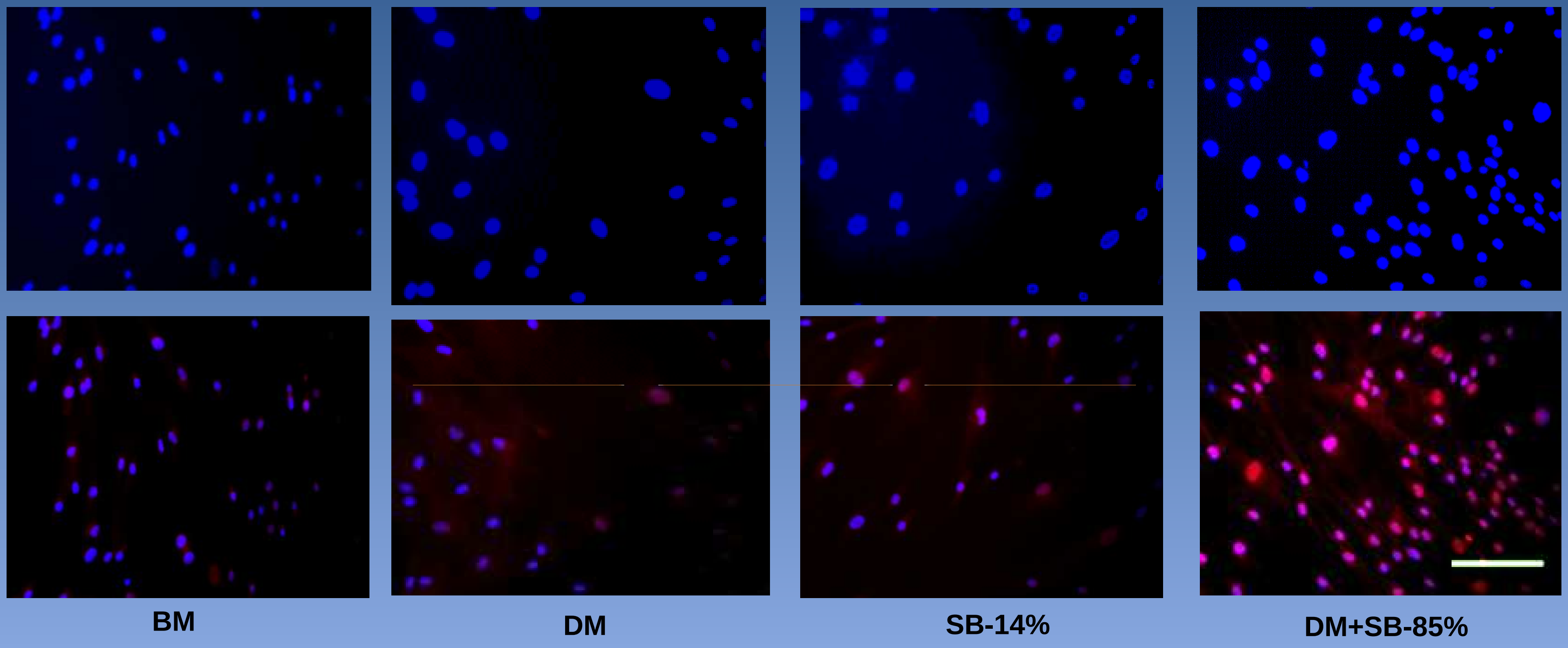


SB-SB431542 TGFβ pathway inhibitor

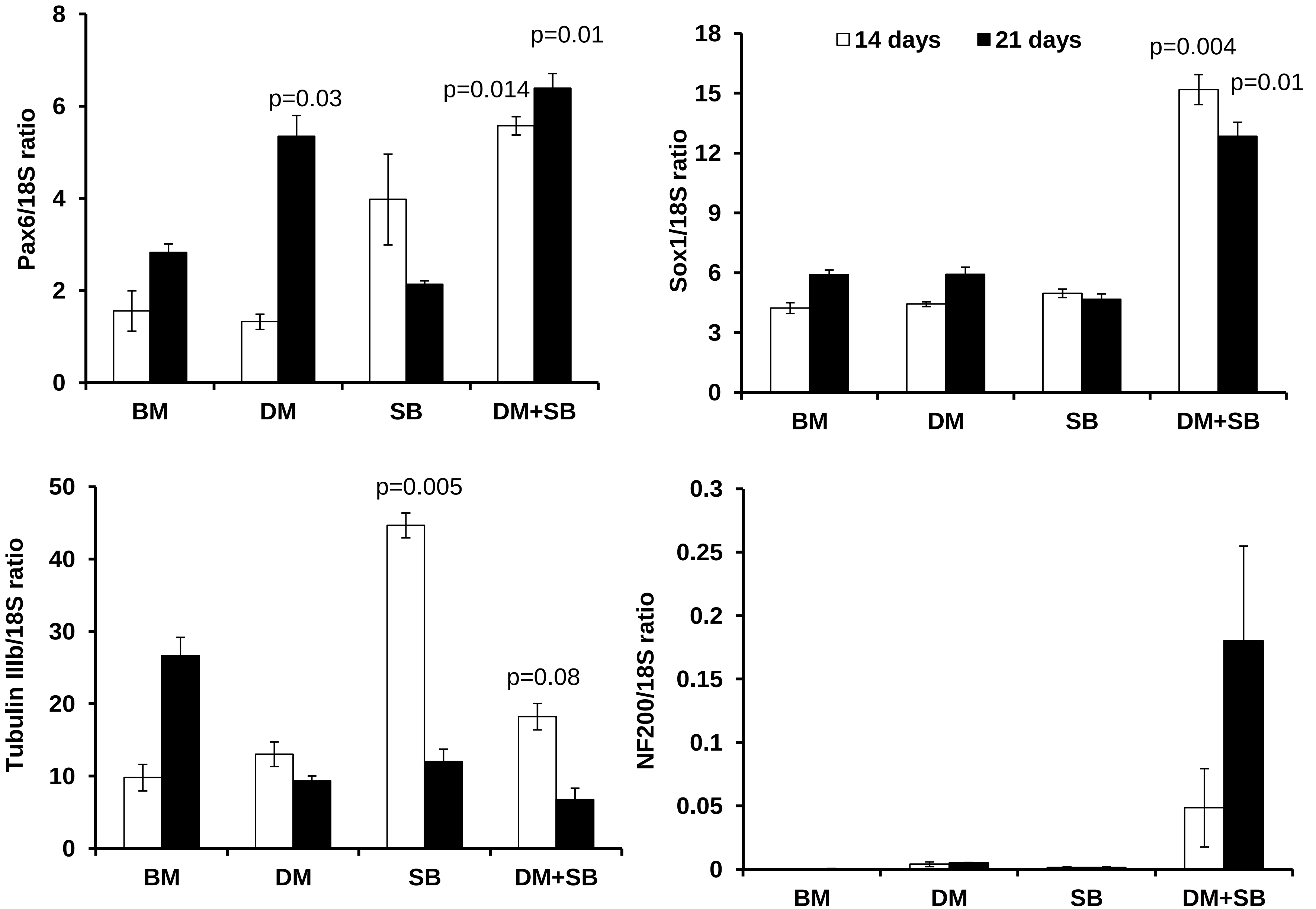


DM+SB

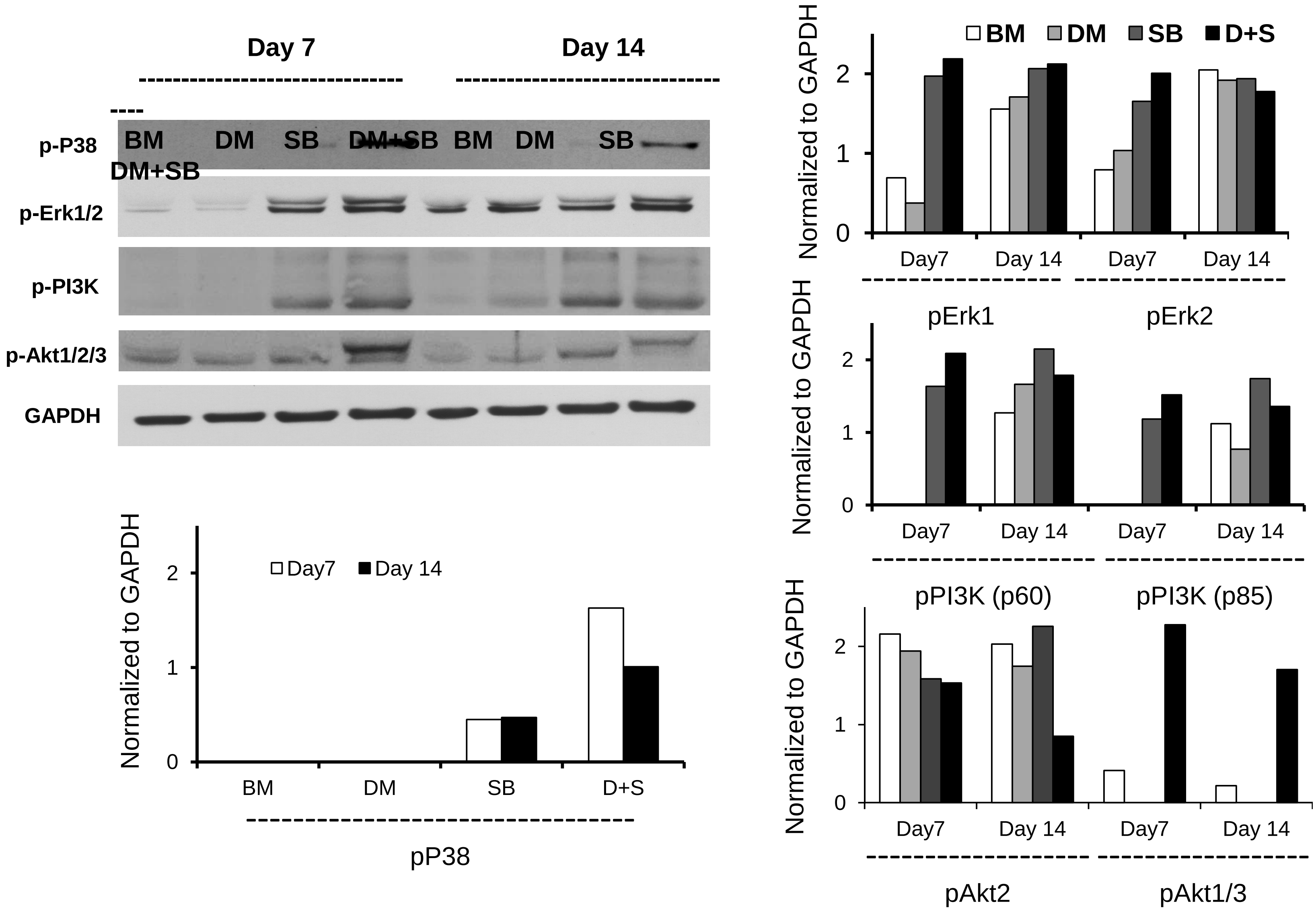
hADSCs express neurite outgrowths with inhibition of TGFβ and BMP pathways-
Length of neurite outgrowths significantly longer in DM+SB group



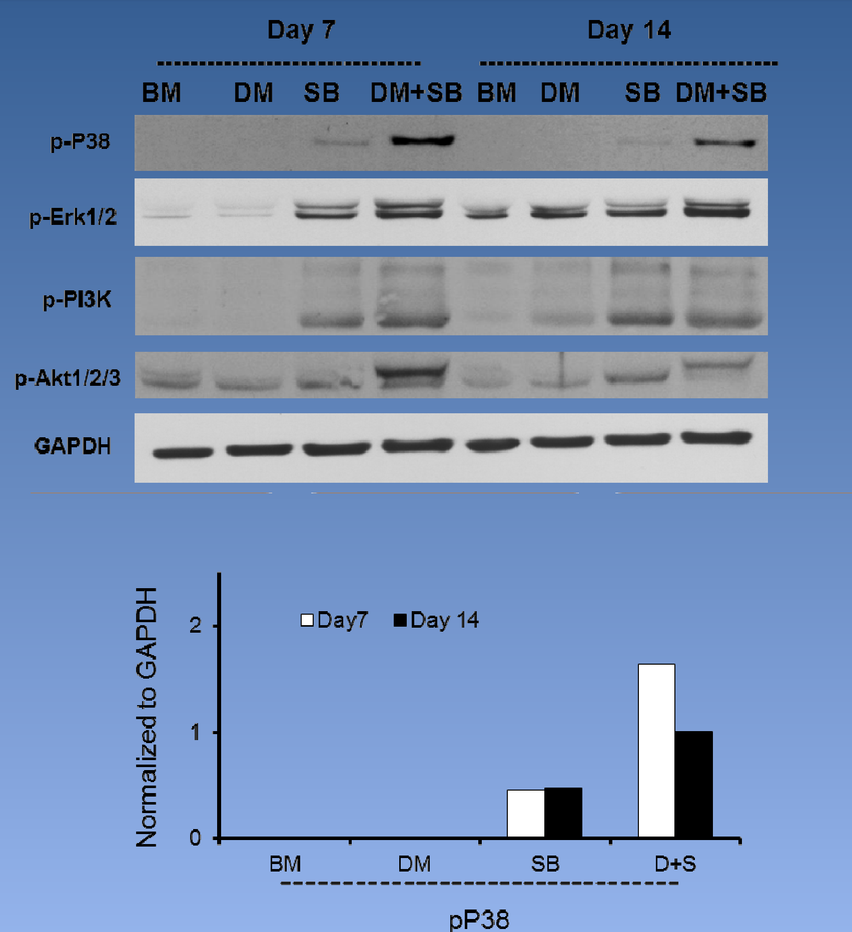
hADSCs express neuron specific enolase upon dual inhibition of TGFβ and BMP inhibition



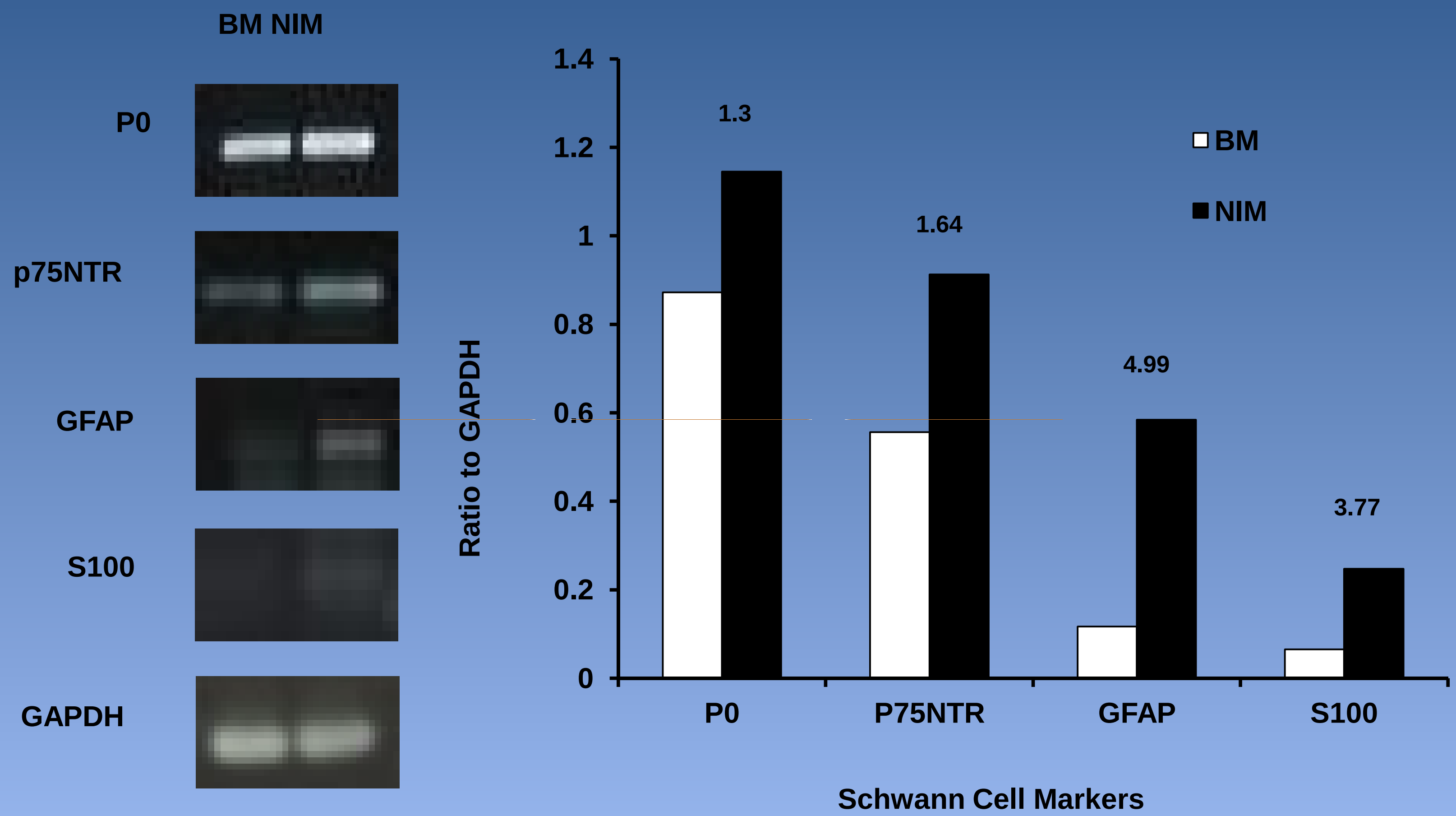
mRNA expression of neuronal markers in hADSCs increases with inhibition of TGFβ and BMP pathways



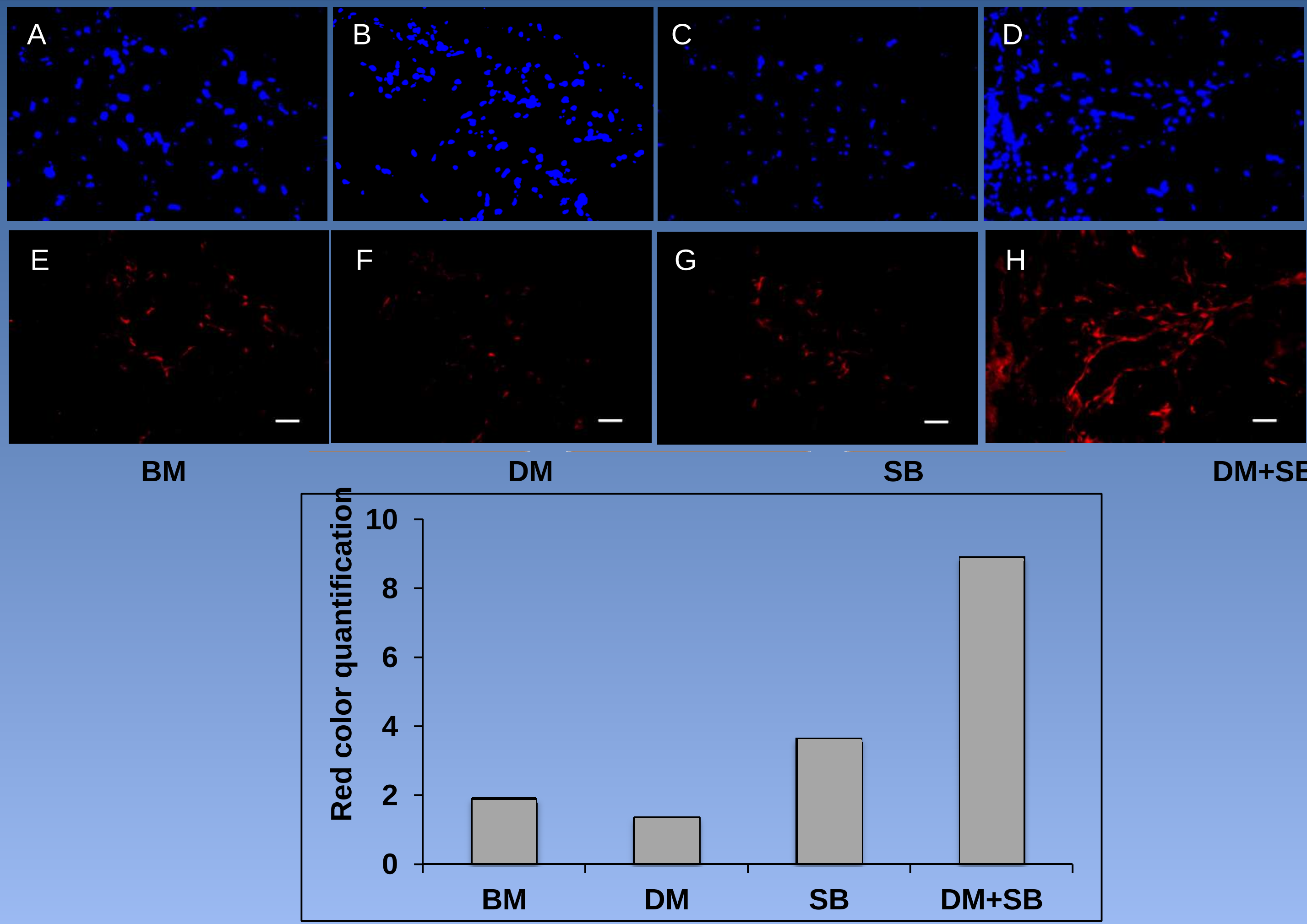
Day 7
Day 14
p-P38
BM DM SB DM+SB BM DM SB DM+SB
p-Erk1/2
p-PI3K
p-Akt1/2/3
GAPDH
Normalized to GAPDH
Day7
Day 14
BM
DM
SB
D+S
pP38
BM DM SB D+S
pErk1
pErk2
pPI3K (p60)
pPI3K (p85)
pAkt2
pAkt1/3



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

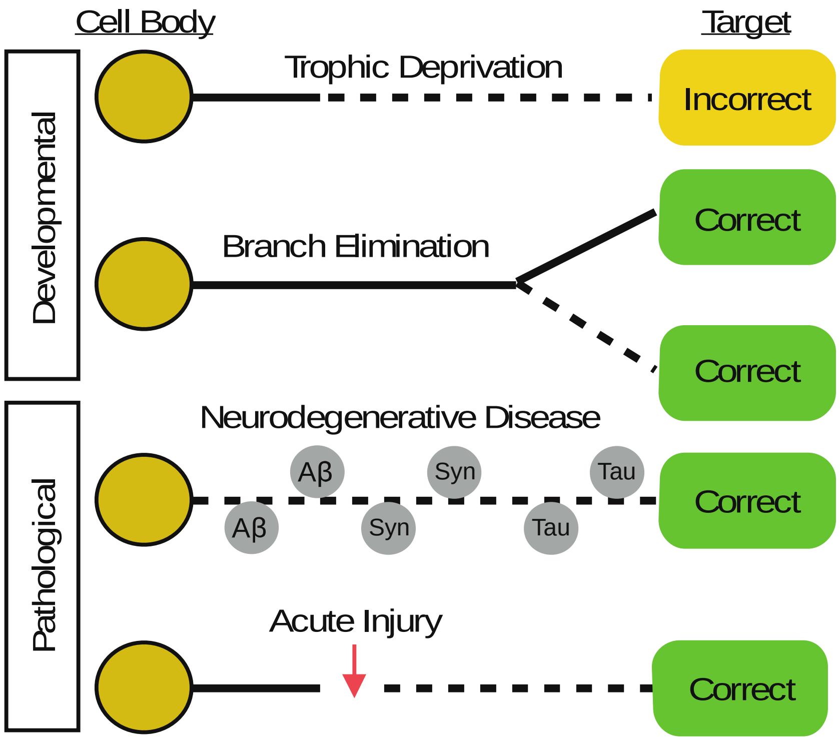
Finally…

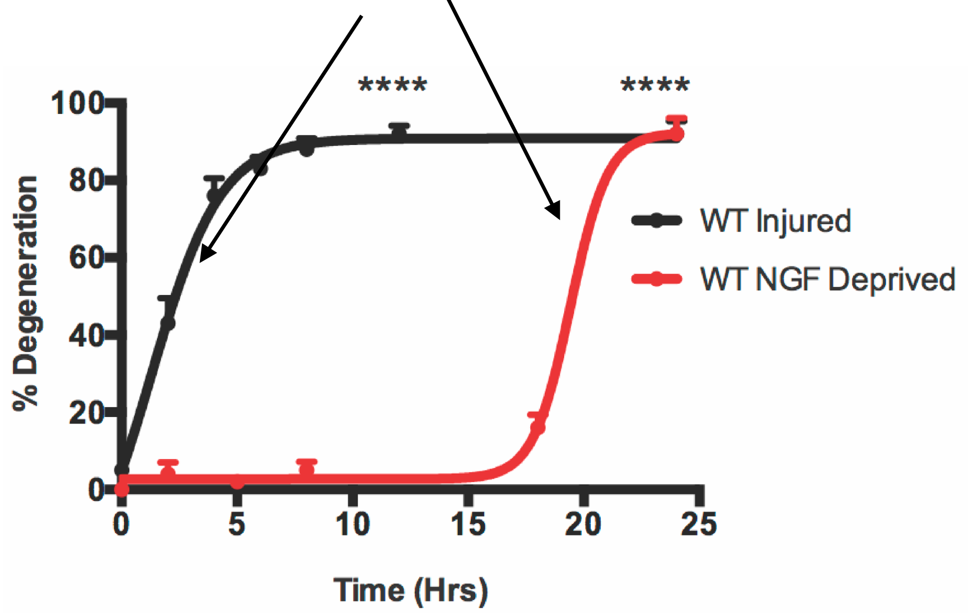
<u>Stem Cells International 2016</u>

Dual Inhibition of Activin/Nodal/TGF-β and BMP Signaling Pathways by SB431542 and Dorsomorphin Induces Neuronal Differentiation of Human Adipose Derived Stem Cells.

Madhu V, Dighe AS, Cui Q, Deal DN.

Christopher Deppmann
Axons coordinate each other's degeneration





- Pathological degeneration has co-opted those used in developmental processes
- This is not a passive, axon autonomous process——role for axons and other cells
- We've begun to identify the molecular mechanisms governing this coordination
- Mechanisms of coordination may explain bystandard degeneration observed in TBI, stroke, and SCI.

Coulter Grant Application 2015

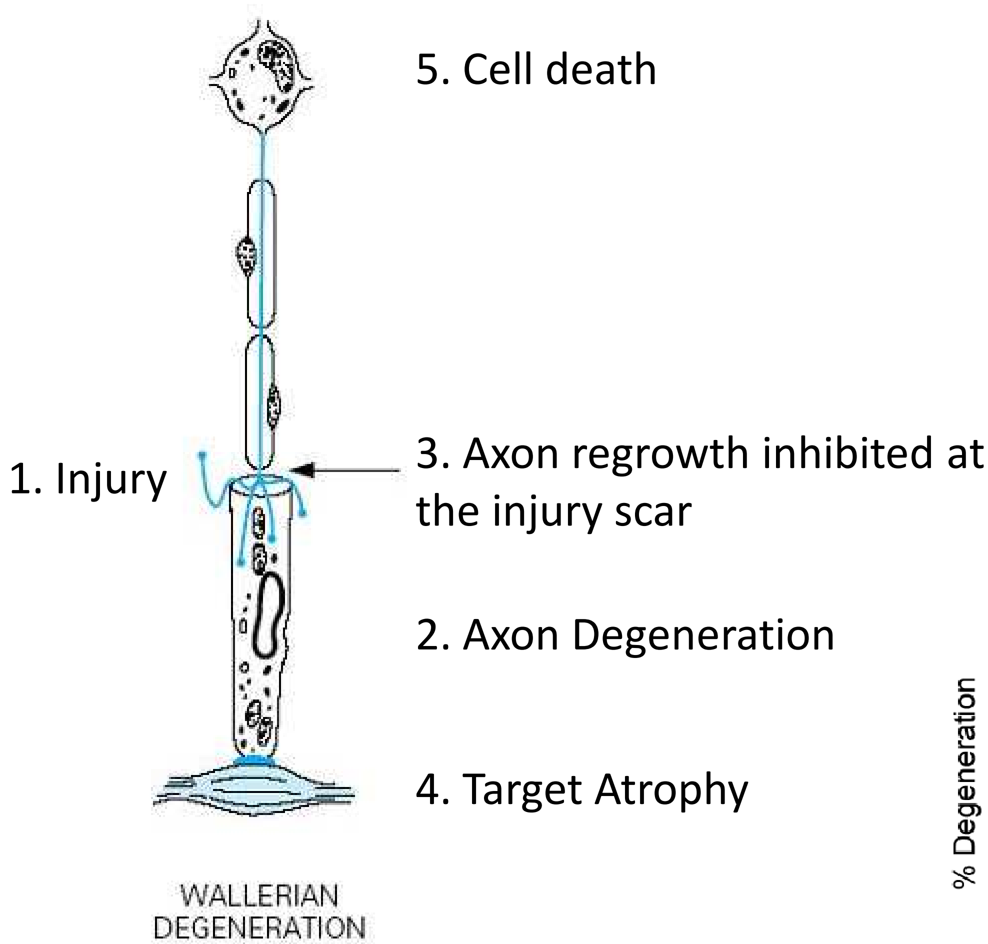
Overview of milestones:

- 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 2016

- **Overview of milestones:**
- Milestone 1 (q1): Examine whether remote neural activity is sufficient to promote regeneration of injured neurons.
- Milestone 2 (q2): Examine whether remote cAMP activity is sufficient to promote regeneration of injured neurons.
- Milestone 3 (q3): Combinatorial testing and development of wearable magnetic stimulators
- Milestone 4 (q4): Move toward testing in a clinical setting.

Approach:
Succeeding where others have failed



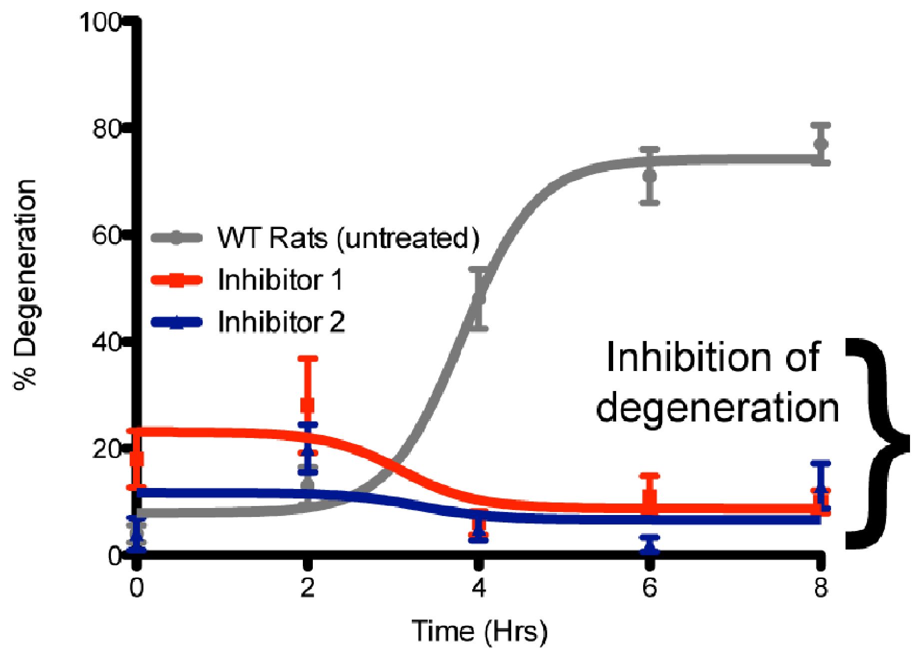
•Path to therapy:

Cell culture→Animal model→Human trials

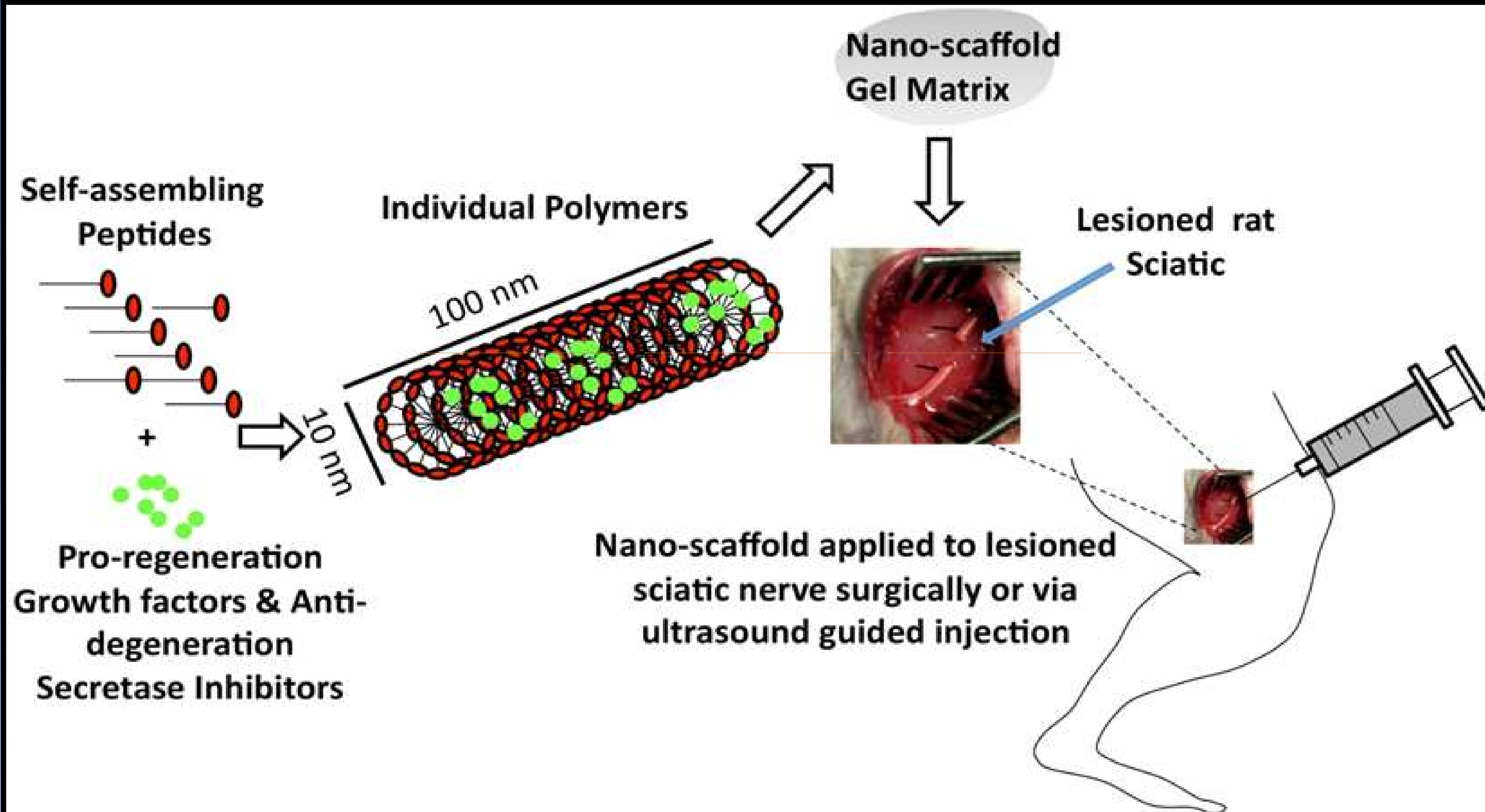
Rapidly deliverable porous scaffold to cross scarred tissue

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

Proprietary compound(s) that prevent axon degeneration

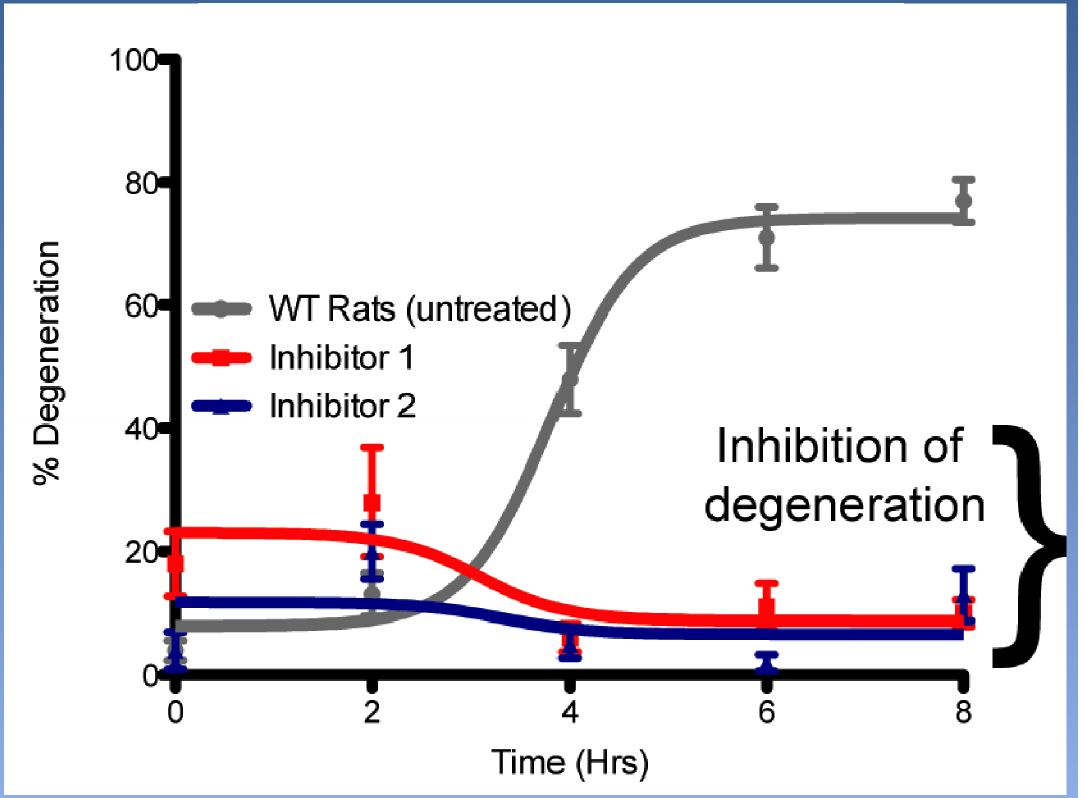


Approach 1: Self forming nano-scaffold for rapid, non-invasive regeneration

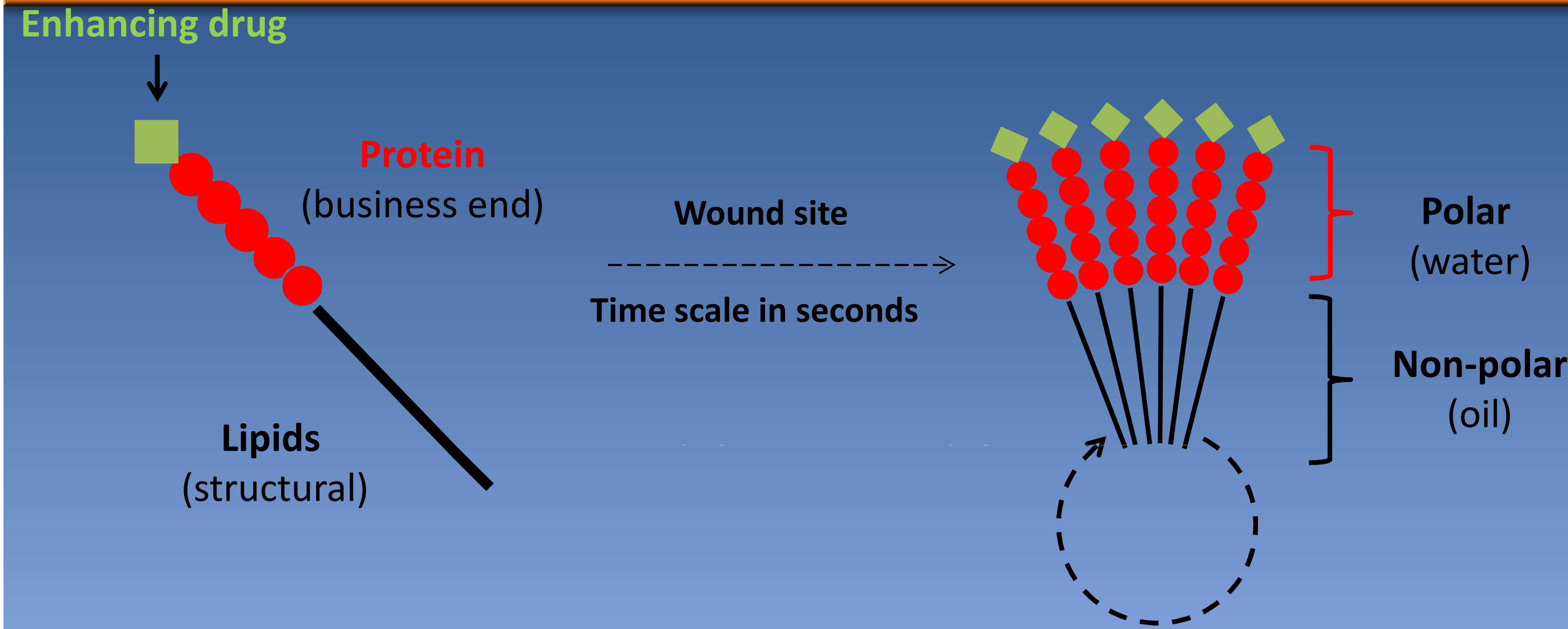


Coulter Grant Application

- 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

Coulter Grant Application

UNIVERSITY of VIRGINIA

•Biocompatible – minimal immune response; biodegradable

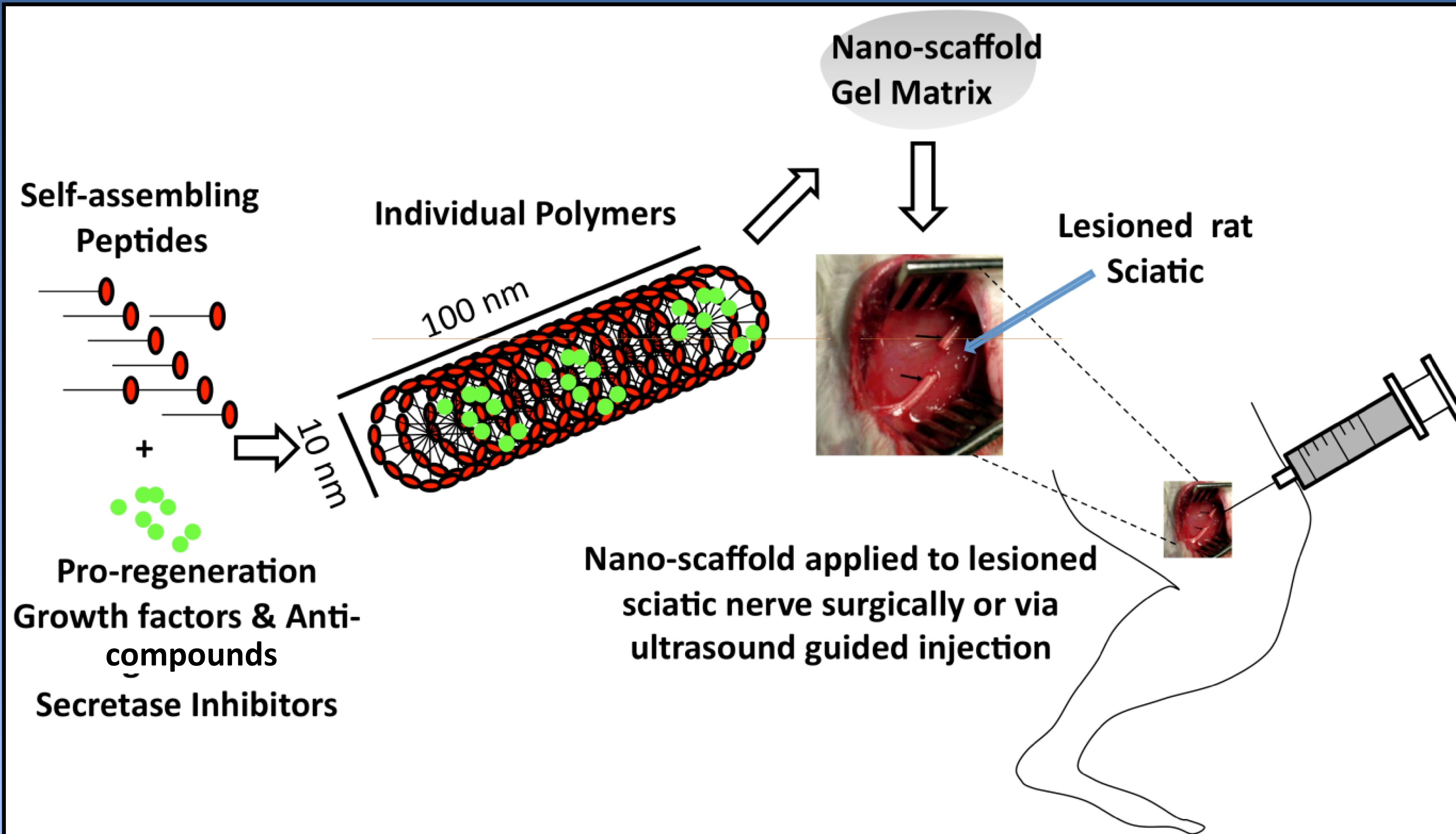
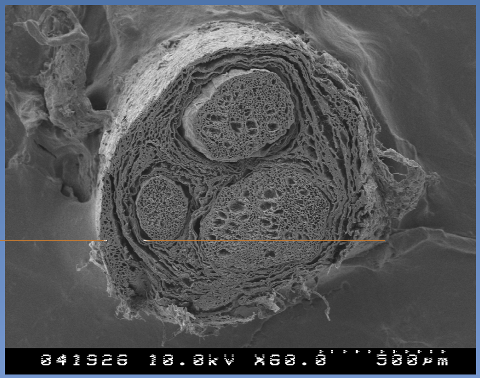
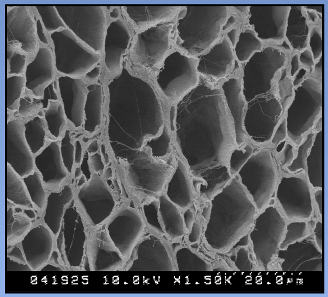


Figure 2: Schematic of Preclinical Strategy for Nerve Repair

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

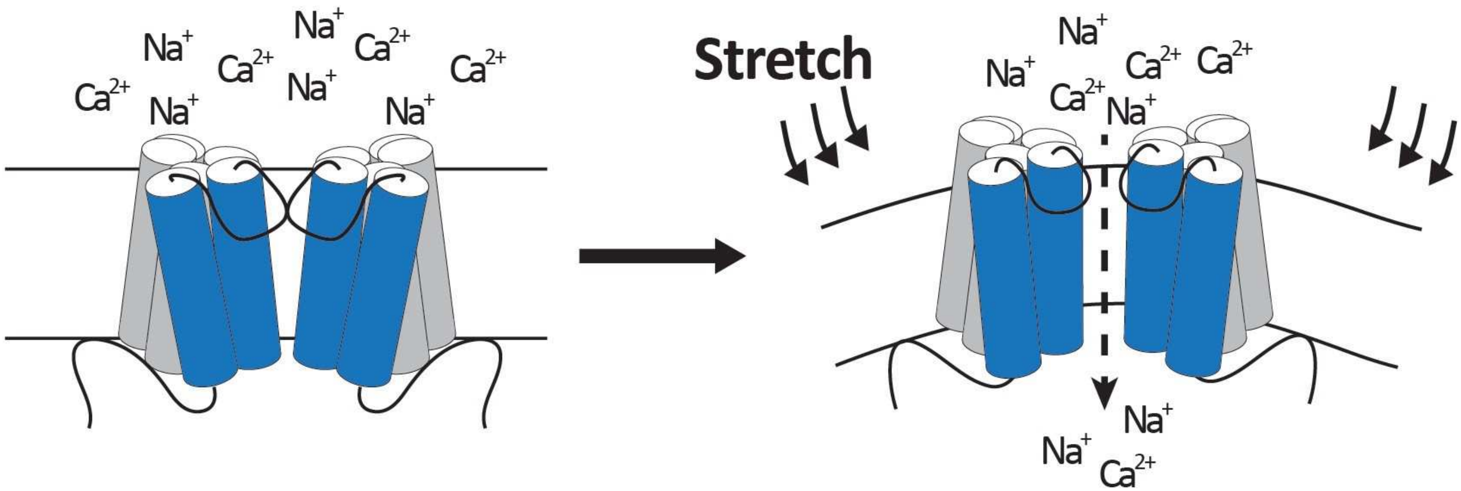
J Hand Surg Am. 2013 Oct;38(10):1965-71.**Allograft reconstruction for digital nerve loss.**
Taras JS[1], Amin N, Patel N, McCabe LA.

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

Approach 2: Using magnetoreceptors for remote nerve repair

TRPV4



Wheeler et al., *Nat. Neurosci.*, 2016

Questions