Preventing Degeneration of the Neuromuscular Junction

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Nerve Injury

A. Normal neuron

B. 2 weeks after injury
   - Fewer Nissl bodies
   - Degenerating fiber and myelin sheath
   - Peripheral nucleus
   - Macrophage

C. 3 weeks after injury
   - Atrophied muscle
   - Proliferating Schwann cells
   - Axon penetrating Schwann cells

D. 3 months after injury
   - Successful nerve regeneration
   - Muscle regeneration

Unsuccessful nerve regeneration

E. Months after injury
   - Disorganized axon growth
   - Atrophied muscle
   - Cord of Schwann cells
The Neuromuscular Junction

Figure 2: The Adult Neuromuscular Junction
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.
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
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
Stem Cells International 2016
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.
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 by standard degeneration observed in TBI, stroke, and SCI.
Overview of milestones:

- **Milestone 1 (q1):** Assess the ability of single compound/nanoscaffold formulations to prevent degeneration, 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.
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

1. Injury
2. Axon Degeneration
3. Axon regrowth inhibited at the injury scar
4. Target Atrophy
5. Cell death

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

Path to therapy:
Cell culture → Animal model → Human trials

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

Self-assembling Peptides

Pro-regeneration Growth factors & Anti-degeneration Secretase Inhibitors

Individual Polymers

100 nm

10 nm

Nano-scaffold Gel Matrix

Lesioned rat Sciatic

Nano-scaffold applied to lesioned sciatic nerve surgically or via ultrasound guided injection
• 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

Enhancing drug

Protein (business end)

Lipids (structural)

Wound site

Time scale in seconds

Polar (water)
Non-polar (oil)

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• Biocompatible – minimal immune response; biodegradable

**Figure 2: Schematic of Preclinical Strategy for Nerve Repair**

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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)
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%

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

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

• 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

Questions