Engineering Approaches for Nervous System Injury and Repair

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Traumatic Nerve Injury

Central Nervous System¹
- 255,000 SCI cases in U.S.
- 12,000 new cases per year
- 90% survive and live near-normal life spans
- Extremely limited therapeutics

Peripheral Nervous System²,³
- 3-5% of all trauma patients
- 90,000 new cases per year
- 50% result in some functional recovery
- Limited therapeutics

**Limited regeneration following trauma**

³Sinai Hospital; http://www.lifebridgehealth.org/Sinai/TraumaFactsandInformation.aspx. Retrieved February 2013; A website with trauma facts and figures.
Peripheral Nerve Trauma

- 360,000 in U.S. suffer from upper extremity paralytic syndromes annually \(^1\)
- Trauma due to stretch, crush, laceration or blast exposure
- 81% upper extremities and 11% lower extremities \(^2\)
- Mild trauma: Minimal sensory and/or motor deficits \(^3\)
- Severe trauma: Major loss of function, intractable neuropathic pain \(^3\)

Anatomy of Peripheral Nerve

Nerve Injury Classification

FIGURE 1. Nerve injury classification. (A) Cross-section of a normal nerve. (B) Illustration of injury classifications. Type I: myelin disruption with axons intact. Type II: axon disruption with intact perineurium. Type III: damaged Schwann cell basal lamina and endoneurial scarring inhibiting regeneration. Type IV: nerve fascicle disruption and loss of the perineurium sheath; repair required. Type V: disruption of the entire nerve; repair required. Type VI: mixed injury of all types along the damaged nerve. Reprinted with permission from Ref. 6.

Repairing Nerves
End-to-End Direct Repair or Neurorrhaphy

Tension restricts epineurial blood flow causing tissue necrosis
Nerve Autologous Graft to Avoid Tension

Nerve Transfer
Regeneration is Slow
Regeneration is Selective
Nervous Tissue Engineering

• Nerve regeneration:
  – Autologous grafts
  – Synthetic nerve guides; what do they do?
  – Schwann cell transplants
  – Growth factor addition
  – Biomaterial scaffold
Nerve Repair Strategy

1. Provide physical scaffold on regeneration can occur
2. A regenerative pathway that persists long enough for axons to reach their target
3. Keep regenerative signaling active until functional recovery is achieved
Nerve Repair Scaffold

Developing a tissue-engineered neural-electrical relay using encapsulated neuronal constructs on conducting polymer fibers

D Kacy Cullen¹, Ankur R Patel², John F Doorish³, Douglas H Smith¹ and Bryan J Pfister¹

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PLA Collagen - PLLA

Nerve Repair Scaffold

Neurite Outgrowth Distance (mm)

0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5

Days

2D

3D

**

**

*

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Control

500 μm
Fibers Provide a Preferred Substrate for Growth

Embryonic, E16 DRG Explants (Rat)
Adult Neuron Outgrowth
Adult DRG and Schwann Cell Co-culture

A. Nuclei

B. NF200

C. S-100b

Merged
Nerve Repair Strategy

1. Provide physical scaffold on regeneration can occur
2. A regenerative pathway that persists long enough for axons to reach their target
3. Keep regenerative signaling active until functional recovery is achieved
Axon Growth: Development of long projection neurons

A six-week-old human embryo (Getty Images)

Chemoattractive & Repulsive Signals:
Netrins, Semaphorins, Ephrins, ECM, CAMs

Order of microns to millimeters
A Long Road Ahead
Axon Growth

Pioneering Axon

Labeled Pathway

Stretch-Induced Axon Growth
Sultan Kosen: 8’ 1”
He Pingping: 2’ 5”
Deer antler sensory axons grow up to 18mm a day!

Gray et. al. 1992

Blue whales grow 40mm a day!

Kato, 1994

A giraffe’s neck can grow by 20mm a day!

Baobabfarm, 2002
Synaptic target
Stretch Induced Injury

Peripheral Nerve Damage

Distension Osteogenesis
Tension restricts epineurial blood flow causing tissue necrosis
Rabbit femur was gradually elongated to 30 mm at the rate of 0.8 mm/day, 2.0 mm/day, and 4.0 mm/day to stretch the sciatic nerve.
Peripheral nerve lengthening by controlled isolated distraction: a new animal model

Markus W. Kroebel a,b,*, Edward Diao a, Shin-Ichi Hida c, Ellen Liebenberg a

a Department of Orthopaedic Surgery, Division of Hand, Upper Extremity, and Microvascular Surgery, and Orthopaedic Research Laboratory, University of California, 500 Parnassus Avenue, Box 0728, San Francisco, CA 94143-0728, USA
b Department of Orthopaedic Surgery, University of Heidelberg, Germany
c Department of Orthopaedic Surgery, Fukuoka University, Fukuoka, Japan

Received 18 June 1999; accepted 13 March 2000

Proximal nerve stump was gradually elongated 1mm/day followed by a direct anastomosis to the distal stump.
Aggressive early treatment (slowing the rate of lengthening and/or performing decompression) allows continued lengthening without incurring permanent nerve injury. When indicated, decompression of the affected nerve should be performed as soon as possible, thereby improving the chances of and shortening the time to complete recovery.
Towed Growth of Axons

Axonal Growth in Response to Experimentally Applied Mechanical Tension

D. Bray

MRC Cell Biophysics Unit, 26-29 Drury Lane, London WC2B 5RL, England
Axon Stretch-Growth

Rat dorsal root ganglion neurons
Fasciculated Axon Tracts

[Image of fasciculated axon tracts with a scale indicating 10 microns and 1 microns.]
Extreme Axon Stretch-Growth

5cm
Nerve Repair Strategy

1. Provide physical scaffold on regeneration can occur
2. A regenerative pathway that persists long enough for axons to reach their target
3. Keep regenerative signaling active until functional recovery is achieved
Nerve construct sized to length of damage

Two potential therapeutic benefits:

1. Establish a living relay across the lesion
2. Enhance regeneration and guide damaged axons across the lesion

Long-Term Survival and Integration of Transplanted Engineered Nervous Tissue Constructs Promotes Peripheral Nerve Regeneration

Transplantable Nerve Construct
Host Construct Integration

GFP

NF200
Host Construct Integration

GFP+  AP+
Post-transplant Myelination

MBP

NF200
Nerve Repair Strategy

1. Provide physical scaffold on regeneration can occur
2. A regenerative pathway that persists long enough for axons to reach their target
3. Keep regenerative signaling active until functional recovery is achieved
Nerve regeneration requires a pathway

1. Schwann Cell forming Bands of Büngner
2. Presence of the degenerating distal nerve
Boundaries of Stretch-Growth
Paradox

Cytoskeletal proteins transport ~ 1mm/day

Axon stretch-growth can reach 10mm/day
Axons Expand In Length And Caliber
Axon Caliber, TEM

36.5% Average; 40.8% Median
Location of Axonal Growth

Percent Change in Length
Stretch Growth of Adult Axons
Human DRG

14 patients – cervical ganglionectomy
4 organ donor – thoracic

Cultures survive at least 3 months
Adult Human DRG Axons

- Stretch-grown to 1cm in length
Axon Stretch Growth

Pfister, Aquino & Loverde - 2010’s

• Designed for study of axon stretch growth

• Led to development of unidirectional / physiological stretching method

• Microscope stage sized

• Closed loop temperature regulation & gas ports for real-time analysis

• No. 1 coverslip bottom for microscopy

• Up to 4 cm axon length
1. DRG PLATING
2. GROWTH CONE EXTENSION
3. STRETCHING
Injury or Stress Response?

Primary injury events lead to secondary injury cascades:
- Positive signals
- Negative signals
- Sustained ion imbalance
- Gene expression changes
- Chromatolysis
  - Cell body swelling
  - Dispersion of Nissl substance
  - Eccentric nuclei

Injury Events*

- Rapid depolarization
  - "injury discharge,"
  - burst of action potentials

- $\uparrow Ca^{2+}$ $\uparrow Na^+$
- Neurotrophin

Poisson Effect

Axon deformation & occlusion due to stretch

Mitochondrial Kymograph

60x
Morphology Study:
Axon deformation & occlusion due to stretch

**Velocity**

- Anterograde
- Retrograde

**Flux**

- Axon #1
- Axon #2
- Axon #3

≥ 25% Strain Impedes Transport (eDRG)
ASG of Dissociated Cells:
“Reverse” seeding for analysis of perikarya

Bar = 100 μm

Bar = 25 μm
Analysis of perikarya for chromatolysis

**Axonal Strain**

Strain (ε)

Day

3mm/d

n = 161
n = 38
n = 216
n = 51

*p < 0.001

18-19 DIV

A

Average Cross-Sectional Area (μm²)

Control
ASG Low Strain Profile
ASG High Strain Profile
Stretch Axotomy Profile

n = 161
n = 38
n = 216
n = 51

*** p < 0.001

B

Bar = 10 μm

18-19 DIV
Electrophysiology
Comparative Injury Study:
Electrophysiological response

During Stretch

Post Stretch

Spontaneous Action Potentials in Rested Versus Acutely Stretched Neurons

Bar = 25 μm
Comparative Injury Study: 
Calcium flux

Fluo4 calcium time-lapse imaging

Normalization Average Intensity

Frame

8 DIV

20 DIV

2 min
5% strain

25 µm
Comparative Injury Study: HSP70 Kda (HSPA5) mRNA smFISH

Phase contrast

Wide-field epifluorescence

Laplacian filtered, Quantified with Matlab

Bar = 5 µm
Microarray Analysis: Optimized main assay

Stretch Growth Optimizations
- Fewer non-stretched cells
- Axons of equal length
- Axons undergo homogenous stretch
- Optimized stretch profile

Control Optimizations
- Sham-style controls on towing substrates
- Matched cervical DRGs
- Bilateral isolation from same embryo

Top 5 of 17 paired experiments selected
- 71 of 71 experimental explants lysed underwent axon stretch growth
- 66 of 72 sham explants lysed crossed the substrate interface and grew as expected
Microarray Analysis:
Analysis of final dataset

Axonal Development Genes

**Diffusible ligands and receptors**

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**Membrane-bound ligands and receptors**

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Growth cone motility in coordination with axon stretch growth. (A) 18 hours elapsed: ASG; arrows indicate non-solubilized PDL aggregates with stretch grown axons attached. (B) 30 hours elapsed; arrows indicate continued growth cone extension in coordination with ASG. (C) 42 hours elapsed; continued growth cone extension evident by confluence of previously non-confluent areas. Scale bar = 500 μm.
**Microarray Analysis:**

**Analysis of final dataset**

<table>
<thead>
<tr>
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<th>Q Value</th>
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**Na\textsubscript{v}1.1 Increase in ASG soma and axons**

**K\textsubscript{v}1.2 Increase in ASG soma**
## Microarray Analysis:
### Analysis of final dataset

**Regenerative Associated Genes**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
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<th>Accession Number</th>
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Microarray Analysis: Drug effect on axon outgrowth

Custom qRT-PCR array plate:

Embryonic axon outgrowth over 3 DIV

Evaluate in central & peripheral neurons
Neural Tissue Engineering

1. Provide physical scaffold on regeneration can occur
2. A regenerative pathway that persists long enough for axons to reach their target
3. Keep regenerative signaling active until functional recovery is achieved
Pfister Laboratory
Center for Injury Bio-mechanics, Materials and Medicine

Lab Members:
Presented work:
Joseph Loverde, PhD
Mevan Siriwardane, PhD

Current Members:
Mohammed Abdul Muneer, PhD
Mathew Long, MS
Alexandra Adams
Joseph Malaro
Brian Swenson
Kyle O’Brien

NEURO- eNgineering REU Site