Neural Biomaterial Interfaces in Tissue Engineering & Regenerative Medicine

Traumatic Injuries of Peripheral and Central Nervous Systems
In Vivo Strategies and In Vitro Pipeline

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Convergence as Tissue Engineering has evolved

Tissue Replacement
cells grown in culture and seeded into a material scaffold

Self assembly
cells grown in culture produce their own matrix or endogenous stem cell stimulation

Tissue Control
material induces specific response in vivo; e.g., nerves, vessels

modified, courtesy of MV Sefton, U. Toronto

Neural Tissue Engineering Strategies: Traumatic Injuries

• Peripheral Nerve Repair Strategies
  • Nerve guidance channels

• Spinal Cord Repair Strategies
  • Regenerative strategies
    • Entubulation strategies
  • Neuroprotective strategies
    • Drug delivery
    • Stem cell strategies
Peripheral Nerve Repair Strategies

- Suture ends together
  - Works well for small or incomplete gaps
  - BUT: Alignment of proximal and distal ends can be problematic
- Autograft
  - Gold Standard for gaps >5mm
  - Contains SC, endoneurial tubes, NTFs
  - Offers cell and endoneurial tubes, surface adhesion molecules
  - BUT: Secondary injury
    - Donor site morbidity, scar, occasional neuroma, pain
    - Insufficient donor tissue availability, length, diameter
    - Non-specific and incomplete reinnervation
- Bioengineered Nerve Grafts

Bioengineered Graft

- Can manipulate:
  - Length
  - Diameter
  - Rigidity
  - Permeability
  - Degradability
  - Interior Surface – chemistry & morphology

- Combination Strategies:
  - Cells, Growth Factors, Peptides, Proteins
Nerve Guidance Channel Strategies

Design Criteria:
- Porous
- Degradable
- Patent (open during repair)
- Provide guidance to regenerating cable
  - Poly(glycolic acid): NeuroRegen
  - Poly(lactide-co-caprolactone): Polyganics
  - Collagen: Integra Life Sciences

Commercial Nerve Guidance Channels

Axonal regeneration over long distances in the limbs
Monkey median nerve repaired by nerve graft or collagen nerve guide (tube)

Archibald et al, J Neurosci, 1995

University of Toronto

Further legitimizes the concept of entubulation repair.
Confirms the median nerve model for clinical research.
Supports the development of bioresorbable nerve guides.

(Journal of Hand Surgery, Jan '97)

Clinical PGA Tube Nerve Repairs

A Randomized Prospective Study of Polyglycolic Acid Conduits for Digital Nerve Reconstruction in Humans

- 98 patients with 136 nerve reconstructions
- 56 controls, 46 tube repairs (gap lengths less than 20 mm) at follow-up 3, 6, 9, 12 months (sensory recovery)
- Overall, similar outcomes in tube and nerve repair groups
- For short gaps (<4 mm), tube repairs were significantly better than end-end nerve repair
- For longer gaps (≥8 mm), tubes comparable to nerve graft, with sub-group analysis ("excellent" results) favoring tube repair group
Nerve guide: length limitations

Reihrup et al., Ann Neurol, 2002. Factors that influence peripheral nerve regeneration: an electrophysiological study of the monkey median nerve

Challenge: Regeneration over long gaps

- Nerve Guidance Channel:
  - Mechanically strong to remain patent (open)
- Guided regeneration
  - Longitudinally-aligned scaffolds
  - Peptide- or protein-modified scaffolds
  - Neurotrophin concentration gradients

Reinforced Tubes Resist Deformation In Vivo

Katayama et al. Biomaterials 2006 27: 505-518
Longitudinal, peptide-modified channels provide guidance in vitro

Primary dorsal root ganglia neurons extend neurites in peptide-modified channels


Designer Scaffolds: advanced lasers and photochemistry result in biochemical channels that guide nerve cells

Focused laser

Laser penetrating hydrogel matrix and producing free sulfhydryl groups

2-nitrobenzyl protected region

Biomolecules modified region


Concentration Gradients of Soluble Factors Provide Guidance *In Vitro*

- NGF concentration gradient of 133ng/ml/mm: axons guided < 7.5 mm
- NGF/NT-3 concentration gradients of 80ng/ml/mm: axons guided < 12.5 mm
- Goal: immobilize concentration gradient to scaffold that fills device

Cao & Shoichet Neuroscience 2001; Cao & Shoichet Neuroscience 2003
Concentration Gradients Provide Guidance in Model Systems

Goal: immobilize concentration gradient in cell-invasive scaffold that fills device

![Graph showing gradients of NGF in p(HEMA) scaffolds](image)

\[y = 0.2273x\]

\[R^2 = 0.992\]

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Synergism Observed only with Gradients of NGF/NT-3

<table>
<thead>
<tr>
<th>Concentration gradient NGF ng/ml/mm</th>
<th>Concentration gradient NT-3 ng/ml/mm</th>
<th>Preferred Direction of Neurite Outgrowth (in degrees) (n&gt;200)</th>
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</tr>
<tr>
<td>310</td>
<td>0</td>
<td>0 +/- 18</td>
</tr>
<tr>
<td>200</td>
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<tr>
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<td>Constant</td>
<td>None</td>
</tr>
<tr>
<td>Constant</td>
<td>200</td>
<td>None</td>
</tr>
</tbody>
</table>

\[\sum i = \frac{\sum \cos \theta}{n}, \sum i = \frac{\sum \sin \theta}{n}, R = \left(C^2 + S^2\right)^{\frac{1}{2}}\]

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Spinal Cord

- Central communication pathway between the brain and the periphery
- Protected by vertebrae

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Spinal Cord Injury Repair Strategies

- Minimize secondary injury:
  - Spinal cord stabilization and decompression
  - Delivery of neuroprotective agents

- Promote / stimulate regeneration:
  - Artificial bridges (nerve guidance channels or scaffolds)
  - Delivery of neurotrophic factors (NGF, BDNF, NT-3, …)
  - Delivery of antibodies for inhibitory molecules (NogoA, …)
  - Cell transplantation (schwann cells, olfactory ensheathing cells, stem cells)

Spinal cord injury repair strategies

- Applying neurotrophins (NT-3, NGF etc.) by direct infusion or injection to promote the regrowth of nerve fibers.
- Bridging the spinal cord lesions using scaffolds providing a permissive environment:
  - peripheral nerve grafts
  - nerve guidance channels
  - stem cell transplantation
- Applying antibodies against myelin molecules to neutralize their inhibitory effect
- Applying enzymes to degrade the inhibitory molecules in glia scar (proteoglycans): CSPG

Nogo66, MAG and OMgp are ligands for the Nogo66 receptor (NgR). They are expressed by oligodendrocytes and bind to NgR.

Ongoing Clinical Trials

- Autologous, activated macrophages
  - 30 patients, some improvement
- Peripheral Nerve Grafts – cord to cord
  - 10 patients, no improvement
- Human Olfactory Ensheathing Glia (fetal and adult) - into cord
  - 160 patients, improvement, on-going
- Schwann Cells
  - 80 patients, improvement, on-going
- Rho Antagonist: Calthrin
  - On-going (BioAxone)
- Anti-NogoA
  - On-going (Novartis)
- Human ES cells differentiated to oligodendrocytes
  - Planning stages (Geron)
- Human Bone Marrow Stem Cells
  - 90 patients, improvement
- Polyethylene glycol
  - Planning stages
- Electrical Stimulation
  - >110 patients, some improvement, on-going

Spinal Cord Injury Models

Acute Lateral Hemisection
- Bi-Chronic Hemisection
- Gliolysis transsection
- Medullary transection
- Complete transection
- Complete transection with stumps
Spinal Cord Injury Repair Strategies

Compression Injury
our goal: to develop an injectable drug delivery vehicle

Transection Injury
our goal: to develop an entubulation strategy

Entubulation Strategy for Transection Injuries

- Channel helps to create an environment that is more permissive to regeneration
- Prevents ingrowth of fibrous tissue from physically blocking the path for regenerating axons
- Guides growing axons in the right direction
- Can be a vehicle for delivery of various therapeutic strategies

Our Entubulation Strategy:
Creating a Permissive Environment for Nerve Regeneration

Overall hypothesis:
3 elements required for nerve guidance
1. Pathway
   ➢ Porous tube
2. Contact-mediated cues
   ➢ Peptide-modified scaffolds
3. Diffusible Cues
   ➢ Growth factor concentration gradients
Polymer Processing
SpinFX: liquid-liquid centrifugal casting

Polyacrylate Tubes
Dextran Tubes
Chitin Tubes


Enhanced Tubes:
Matrix + Neurotrophic Factor + Tissue Graft

Tubes alone: evidence of regeneration, but limited functional recovery

Enhanced Tubes (n=12/group):
1. Tube + Fibrin Glue + FGF1 + Peripheral Nerves
2. Tube + Fibrin Glue + Peripheral Nerves
3. Tube + Fibrin Glue + FGF1
4. Tube + Fibrin Glue

Tsai, et al. J. Neurotrauma 2004; Tsai, Biomaterials 2006

Hypothesis: advanced device design required for better recovery
Drug-eluting Tubes
Biodegradable Chitin - Chitosan Tubes
Incorporate PLGA Microspheres

Microspheres incorporated between chitin outer and chitosan inner layers
Microspheres incorporated in chitin outer layer that surrounds chitosan inner layer

Injectable Delivery Vehicle for Compression Injury Repair

Healthy Spinal Cord
Injured Spinal Cord

1. Intervertebral disc
2. Vertebral body
3. Dura mater
4. Extradural or epidural space
5. Spinal cord
6. Intrathecal space (SAS)

Conventional Intrathecal Drug Delivery

- Acute IT injection
  - Transcutaneous injection to the SAS
- Continuous IT delivery
  - Delivery of chronic pain and antispasticity agents via a minipump

Limitations
- Potential for infection
- Invasive
- Possible inflammatory reaction
- Delivery is not localized
- Long-term delivery is difficult to manage
Injectable Drug Delivery Vehicle
minimally invasive & localized release

Design Criteria for 
Drug Delivery System

- Localized delivery of therapeutic agents
  - Avoid systemic side effects
  - Decrease dosage administered
- Minimally invasive system
  - Biocompatible
  - Biodegradable
  - Fast gelling
  - Non-adhesive to cells

Hyaluronan / Methylcellulose

HA
Shear Thinning

MC
Thermal Gelling

Gelation temperature: 18 °C
New Delivery Strategy is Safe
Injection of HAMC vs. aCSF

- Healthy spinal cord
- Injured spinal cord

Hydrodelineation / hydrodissection needles

Dura Resealing

- aCSF
- HAMC

Inflammation

- Histogram showing comparison between aCSF and HAMC

n = 6; * p < 0.05
**Functional Behaviour – BBB Scale**

- HAMC Uninjured
- aCSF Uninjured
- HAMC Injured
- aCSF Injured

Days after surgery:
- Days 0, 1, 3, 6, 9, 12, 15, 18, 21

**HAMC Injured**

**HAMC Uninjured**

**aCSF Injured**

**aCSF Uninjured**

- n = 8; * p<0.05

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**rhEGF: Deep Penetration of Injured Cord**

- 30 minutes
- 6 hours
- 1 day
- 7 days

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**EGF and FGF-2: Undetected in Spinal Cord after Intrathecal Bolus Injection**

- EGF
- FGF-2
Injectable Strategy Shows Promise

- Localized release of factors delivered by intrathecal injection in fast gelling polymers
- EGF localized in injured cord at site of injection
- Some therapeutic benefit
  - cavitation, ependymal cell proliferation
- Novel injectable gels:
  - HAMC has some functional benefit
- Broad Applicability to Neurological Disorders:
  - Traumatic Brain Injury
  - Stroke

Challenges for SCI Repair: No Magic Bullet

Combination Strategies:
- Neuroprotective & Neuroregenerative Strategies
  - What to deliver?
    - Anti-inhibitory factors: NogoA, Cethrin?
    - Myelinating cells: oligodendrocytes?
    - Chondroitinase abc?
    - Tissue Engineered Structures?
  - When to deliver?
    - Biomimetic strategies of development?
  - Where to deliver?
    - Injury site
    - Above / below site of injury
- Animal Models of Chronic Injury

Tessier-Lavigne & Goodman, Science 1996