Spinal Cord Injury Transplants
Autologous Schwann Cell Transplantation for SCI Repair

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EPIDEMIOLOGY OF SPINAL CORD INJURY (SCI)

• **Prevalence:** 253,000 individuals in the U.S. (2006 data, NSCISC)  
  1.25 million (2009 data, CDRF)

• **Incidence:** ~ 12,000 new cases/year in the U.S. (CDC unpublished data, NCICP)

• **Who’s at risk:** Since 2000 (NSCISC) --  
  77.8% of SCIs occur among males

  63.0% are Caucasian, 22.7% are African American, 11.8% are Hispanic, and 2.4% are from other racial/ethnic groups

  Average age at injury is 38.0 years
**Etiology:**

Etiology of SCI Since 2000

- Sports: 8.7%
- Falls: 23.7%
- Violence: 13.7%
- Other/Unkn.: 7.0%
- Vehic. Crashes: 46.9%

**Costs:**

Nationally an estimated $9.7 billion each year (Berkowitz 1998)

<table>
<thead>
<tr>
<th>Severity of Injury</th>
<th>Average Yearly Expenses (in May 2006 dollars)</th>
<th>Estimated Lifetime Costs by Age At Injury (discounted at 2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Year</td>
<td>Each Subsequent Year</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>High Tetraplegia (C1–C4)</td>
<td>$741,425</td>
<td>$132,807</td>
</tr>
<tr>
<td>Low Tetraplegia (C5–C8)</td>
<td>$478,782</td>
<td>$54,400</td>
</tr>
<tr>
<td>Paraplegia</td>
<td>$270,913</td>
<td>$27,568</td>
</tr>
<tr>
<td>Incomplete Motor Functional at Any Level</td>
<td>$218,504</td>
<td>$15,313</td>
</tr>
</tbody>
</table>
**Spinal Cord Injury Pathophysiology**

- Injury - tissue loss, cyst formation, glial scar, abortive repair
- Need to prevent tissue loss / replace neural tissue / promote neuro-plasticity and -regeneration
- Neuro-protective, -replacement and –regenerative therapies
TARGETS FOR REPAIRING THE INJURED SPINAL CORD

STIMULATING ENDOGENOUS CELL REPAIR

REMYELINATION REPAIR

MODIFYING THE INTRINSIC ABILITY OF NEURONS TO REGENERATE

SUPPLYING GROWTH FACTORS/CYTOKINES TO STIMULATE AXON GROWTH

REDUCING THE INHIBITORY ENVIRONMENT/SCARRING

NEUROPROTECTION/IMMUNOMODULATION

http://neuro-surgery-research.academia.edu/
CELLULAR THERAPIES FOR SCI REPAIR

- Fetal nervous tissue
- Peripheral nerves
- Fibroblasts
- Immune cells (macrophages/dendritic cells)
- Schwann cells (SCs)
- Olfactory ensheathing glia (OEG)
- Bone marrow stromal cells
- Embryonic/fetal neural stem and progenitor cells
• Easy to harvest, grow in large quantities
• Do not produce further damage
• Survive in the injured cord, do not form tumors
• Limited bio-distribution
• Replace lost cells directly (nerve cells) or structurally
• Support nerve fiber growth or insulate them
• Improve functional outcomes
• Do not cause aberrant sensations or pain
Schwann Cells for SCI Repair: Functional Properties

- Support regeneration of PN axons
- Secrete growth factors
- Produce growth supportive extracellular matrix
- Myelinate axons
- Autologously transplanted (no immunosuppressants)
REPARATIVE EFFICACY OF SCHWANN CELLS AFTER SCI

Complete & incomplete, thoracic & cervical, sub-acute & chronic experimental SCI models
Incomplete Rat SCI Model: Mascis Contusion at the Thoracic Level

- Clinically relevant: up to 40% injuries
- Pathological changes well documented
- Injury evolution similar in animal models
- Injury models are reproducible
15-20% of SCs survive after spinal cord implantation and SC loss is maximal by 3 weeks post-transplant.

Pearse et al., 2007, *Glia*
SCs do not migrate into the normal host tissue (astrocytic barrier) but remain confined to the implantation site and surrounding penumbra region.
3D ULTRAMICROSCOPY OF CELL IMPLANTS IN THE SUB-ACUTELY CONTUSED SPINAL CORD
Schwann cell grafts prevent tissue loss and cavitation after SCI
**SCHWANN CELLS FOR SCI REPAIR:**

**AXON GROWTH**

Large numbers of axons (red) are found within Schwann cell grafts (green cells) when they are placed into the acutely injured thoracic spinal cord

Pearse et al., 2007, *Glia*

Not only spinal cord axons, but brainstem axons (red) grow into Schwann cell grafts (green cells) when they are transplanted into the chronically injured spinal cord

Barakat et al., 2005, *J Cell Transplant*

Brainstem axons (red) also enter Schwann cell grafts (green cells) when they are placed into the acutely injured cervical spinal cord

Schaal et al., 2007, *J Cell Transplant*
Many axons myelinated (red, red arrows) by implanted Schwann cells (green cells, asterisk) are found within the injured spinal cord.

Pearse et al., 2007, *Glia*
LOCOMOTOR OUTCOMES: OPEN-FIELD LOCOMOTION [BBB SCORE]

MODERATE SPINAL CORD CONTUSION

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No observable hindlimb (HL) movement</td>
</tr>
<tr>
<td>1</td>
<td>Slight movement of one or two joints, usually the hip and/or knee</td>
</tr>
<tr>
<td>2</td>
<td>Extensive movement of one joint or extensive movement of one joint and slight movement of one other joint</td>
</tr>
<tr>
<td>3</td>
<td>Extensive movement of two joints</td>
</tr>
<tr>
<td>4</td>
<td>Slight movement of all three joints of the HL</td>
</tr>
<tr>
<td>5</td>
<td>Slight movement of two joints and extensive movement of the third</td>
</tr>
<tr>
<td>6</td>
<td>Extensive movement of two joints and slight movement of the third</td>
</tr>
<tr>
<td>7</td>
<td>Extensive movement of all three joints of the HL</td>
</tr>
<tr>
<td>8</td>
<td>Sweeping with no weight support or plantar placement of the paw with no weight support</td>
</tr>
<tr>
<td>9</td>
<td>Plantar placement of the paw with weight support in stance only (i.e., when stationary) or occasional, frequent, or consistent weight-supported dorsal stepping and no plantar stepping</td>
</tr>
<tr>
<td>10</td>
<td>Occasional weight-supported plantar steps, no forelimb (FL–HL) coordination</td>
</tr>
<tr>
<td>11</td>
<td>Frequent to consistent weight-supported plantar steps and no FL–HL coordination</td>
</tr>
<tr>
<td>12</td>
<td>Frequent to consistent weight-supported plantar steps and occasional FL–HL coordination</td>
</tr>
<tr>
<td>13</td>
<td>Frequent to consistent weight-supported plantar steps and frequent FL–HL coordination</td>
</tr>
<tr>
<td>14</td>
<td>Consistent weight-supported plantar steps, consistent FL–HL coordination, and predominant paw position during locomotion is rotated (internally or externally) when it makes initial contact with the surface as well as just before it is lifted off at the end of stance, or frequent</td>
</tr>
</tbody>
</table>

Basso et al., Journal of Neurotrauma, 1995
SCHWANN CELLS FOR SCI REPAIR: FUNCTIONAL IMPROVEMENTS

OPEN-FIELD LOCOMOTION: BBB SCORE

INJURY ONLY INJURY with SCs

SELF-SUPPORTED HANGING TEST

INJURY ONLY

INJURY with SCs
SCHWANN CELLS FOR SCI REPAIR: CLINICAL TRANSLATION

- In an immune compromised animal (nude rat with GM1 antibody) examine long-term (6 months) engraftment of the human cell product

- Examined the human SC product in an immunocompromized nude rat SCI paradigm
- Cells used from organ or cadaveric donors, either fresh or cryopreserved. Multiple donors used for each time-point [3 days, 3 weeks, 1.5 and 6 months]
- Cell persistence (survival), proliferation, tumorigenicity, bio-distribution, integration, host responses (scarring, immune response, axon growth), myelination and vital organ pathology examined

ATHYMIC NUDE RAT
EXPERIMENTAL MODELING OF HUMAN SCI

Provided by Kyle Padgett PhD

Normal

Injured

Rodent

Human

B. DOBKRIN, L. HAVTON/ UCLA NEUROLOGIC REHABILITATION PROGRAM
Determine optimal injection methods and cell dose effects in a large animal SCI model.

**Large Animal Studies**
- Thoracic SCI contusion
- Compare stereotactic methods of injection
- Test optimal and high doses of SCs (incl. autologous) & medium volumes for signs of dose toxicity
- Histological, electrophysiological, behavioral and MRI outcomes
SCHWANN CELLS FOR SCI REPAIR:
CLINICAL TRANSLATION

Courtesy, Dr. Guest
• Improved walking ability in moderate-severe acute & chronic thoracic spinal cord injury

• Enhanced upper body strength following acute, cervical spinal cord contusion

• Did not enhance pain after acute or chronic spinal cord injury, are not tumorigenic nor exhibit extensive CNS migration
PHASE 1 CLINICAL TRIALS USING SCHWANN CELLS
Specific Aims

1. Conduct a Phase I clinical trial to determine whether or not there are any toxicities/adverse effects produced by injecting the patient’s own SCs into the spinal cord lesion.

2. Collect Safety (and efficacy) data from 8 patients with complete (ASIA-A) using a dose-escalation design [1x5 million SCs, 2x10 million SCs, 4x15 million SCs] with SCs given at ~1 mo post-SCI; data used for a Phase II randomized clinical trials.

3. Phase 1 safety studies will initially focus on acute SCI patients though protocol amendments will be used for subsequent Phase I/II trials in both incomplete and chronic SCI patients.
SCHWANN CELLS WILL FORM A FOUNDATION FOR FUTURE CLINICAL TRIALS

- Neuroprotection (Rolipram, IL-10, Ephrins, Hypothermia]
- Structural support (biomaterials)
- Growth/inhibitory factors (neurotrophins, cAMP elevation, PSA, klf or PTEN inhibitors)
- Rehabilitation—retraining the injured CNS
- Neural stimulation, neural prostheses
IMPROVING MIGRATION: SURFACE MODIFICATION OF SCs

U. Rutishauser
A. El Maaarouf
M. Ghosh
L.M. Tuesta
R. Puentes
S. Patel
D.D. Pearse
Occurrence: Embryonic tissues, brain regions undergoing plasticity.
Structure: Linear polymer of N-acetylated neuraminic acid in α-2,8-linkage. Added to N-glycan of glycoproteins by the enzyme polysialyl transferase (PST) as a post-translational modification in the Golgi apparatus.
Function: Acts as a permissive agent for cell migration, axon guidance, path finding, axonal sprouting, neurite outgrowth and synaptic plasticity.

Polysialyl transferase

Isozymes: STX and PST
59% identity at the AA level, share enzymatic function.
STX (Expression, embryonic);
PST (Expression, adult tissue)
Intraspinal injections of adenoviral vectors expressing PST promotes corticospinal tract axon growth after dorsal hemisection SCI.

STX expressing Schwann cells are able to intermingle with astrocytes (normally do not) when used in a SC:astrocyte confrontation assay in culture.
GENETIC MODIFICATION OF SCs TO PRODUCE POLYSIALIC TRANSFERASE

To promote PSA surface expression, coding region of mouse PST fused with YFP [N terminal; PST ORF 1080bp] was sub-cloned into the self-inactivating lentiviral vector (LV; pRRLsinPPT-mPST YFP).

LV PST:YFP infected SCs used for implantation

Schwann cells (Passage 1) infected with LV PST-YFP fusion (MOI 50). Maximal expression was observed 96 h post infection. A high infection rate (80-90% SCs) was obtained.
PST-SCs PRODUCE HIGH LEVELS OF POLYSIALIC ACID

Expression of NCAM-PSA on genetically modified Schwann cells

PST-SC    Endo-N treatment    GFP-SC

PSA 150kDa
B-actin 42kDa

Expression of NCAM-PSA on genetically modified Schwann cells

LV-GFP
LV-PST
LV-PST + ENDO N

PSA Immunoblotting on Schwann cells (Arbitrary Density Values)

LV-GFP
LV-PST
LV-PST + ENDO N

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PST-SCs INTEGRATE BETTER INTO THE HOST SPINAL CORD

Migratory front

GFP-YFP:PST-Expressing Schwann cells

GFP-Expressing Schwann cells

(Magnification 10X)
PST-YFP expressing-SCs display significant migration (average up to 8mm) from the site of cell implantation/injury and intermingling with GFAP+ astrocytes.
PST-SCs SUPPORT EXTENSIVE SEROTONERGIC AXON GROWTH
CORTICOSPINAL AXONS LEAVE PST-SC IMPLANTS INTO THE CAUDAL CORD

**A**

GFP-PST SC CST

**B**

<table>
<thead>
<tr>
<th>Distance (μm)</th>
<th>GFP SC</th>
<th>GFP-PST SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-500</td>
<td><img src="#" alt="Graph Data" /></td>
<td><img src="#" alt="Graph Data" /></td>
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<tr>
<td>500-1000</td>
<td><img src="#" alt="Graph Data" /></td>
<td><img src="#" alt="Graph Data" /></td>
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<tr>
<td>1000-1500</td>
<td><img src="#" alt="Graph Data" /></td>
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DISTANCE FROM THE CAUDAL EDGE OF THE LESION
PST MODICATION OF SCs FURTHER IMPROVES FUNCTIONAL OUTCOME

**OPEN-FIELD LOCOMOTOR ABILITY [BBB SCORE]**

![Graph showing BBB Score (0-21 scale) over weeks post-injury for GFP SCs and PST GFP SCs with error bars.]

**FOOT POSITIONING [GRIDWALK TEST]**

![Graph showing number of footfall errors (1/20) over weeks post-injury for various time points with error bars.]

**OPEN-FIELD LOCOMOTOR ABILITY [BBB SUBSCORE]**

![Graph showing BBB Sub-score (0-13 scale) over weeks post-injury with error bars.]

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*Statistical significance indicated by asterisks: * for p < 0.05, ** for p < 0.01, *** for p < 0.001.*
PST-SC MIGRATION & CST AXON GROWTH CORRELATE TO FUNCTION

A

R² = 0.4705*

B

R² = 0.5712*

NO. FOOTFALL ERRORS AT ENDPOINT

MAXIMUM SC MIGRATION IN THE CAUDAL CORD (mm)

NO. FOOTFALL ERRORS AT ENDPOINT

CST AXONS IN THE CAUDAL CORD/mm²
### Supervision/collaboration
- W. Dalton Dietrich PhD
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- Patrick M. Wood, PhD & Wood Lab
- Jim Guest MD PhD
- Alan Levi MD PhD
- Kim Anderson PhD
- Anil Lalwani
- Urs Rutishauer PhD
- Abderrhaman El Maarouf PhD
- Hans-Ulrich Dodt MD
- Nina Jahrling PhD

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- Daniela Garcia-Castillo

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- Denise Koivisto
- Maritza Garcia
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- Rocio Puentes

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- Wei Chen
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- Gravil Joseph
- Dominic Maggio
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- Samik Patel
- Drew Bleicher
- Devin Bustin
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