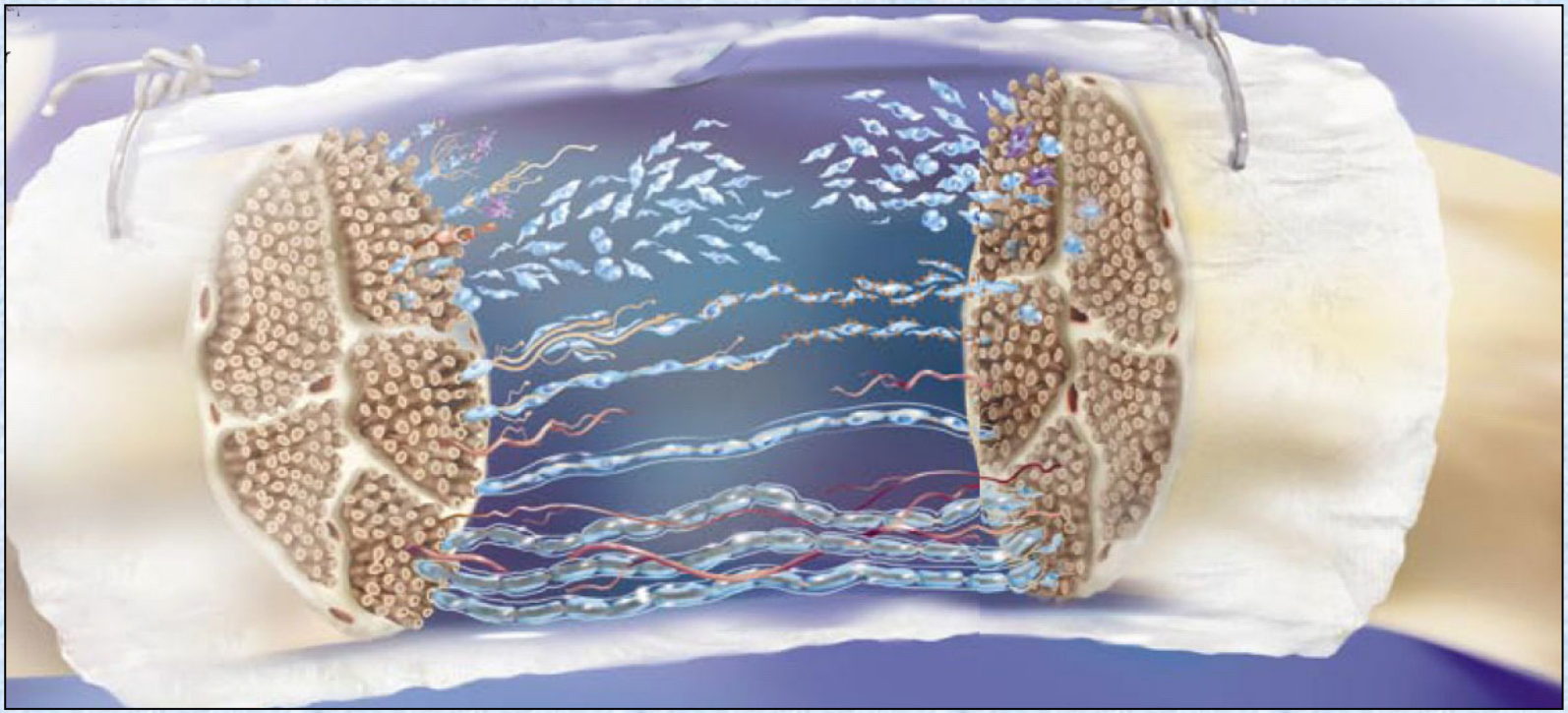
Biomaterials for Nerve Repair: Nerve Guides

Carlos Figueroa and Alex Koculym



[1] Adapted

Introduction

- Statistics:
  - Each Year in the USA:
  - 360 000 people sustain peripheral nerve injuries
  - 10 000 people sustain spinal injuries
  - Globally: 2.8% of trauma patients each year sustain damage to their peripheral nerves **[2] [3]**

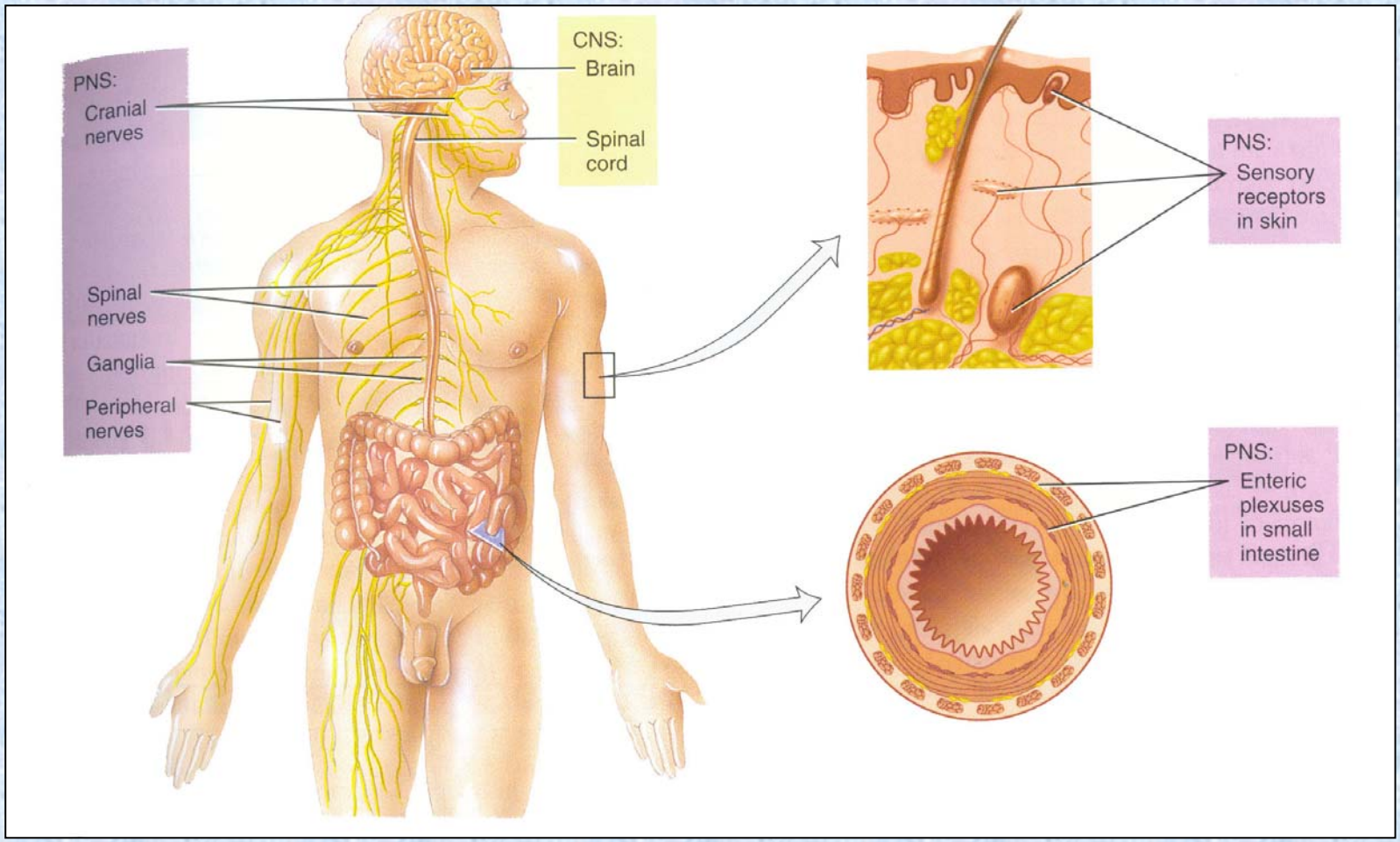
- These injuries can lead to paralysis, loss of function, and chronic pain
- Overall decrease in quality of life

- This presentation will:
  - Introduce Structures in the Peripheral, and Central Nervous Systems
  - Explain some current methods and biomaterials used to repair nerves
  - Examine emerging methods and biomaterials

Enter: The Nervous System

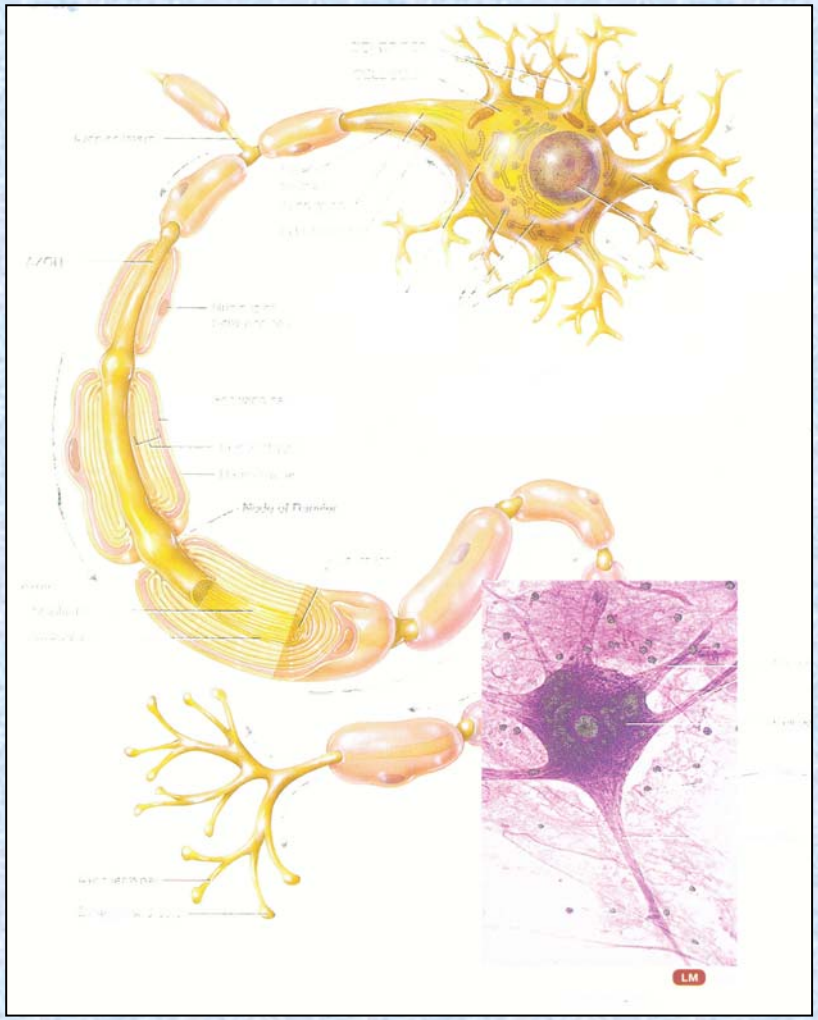
Organization of the Nervous System

The Nervous system is divided into two main subsystems



[4]

Cells of the Nervous System: Neurons

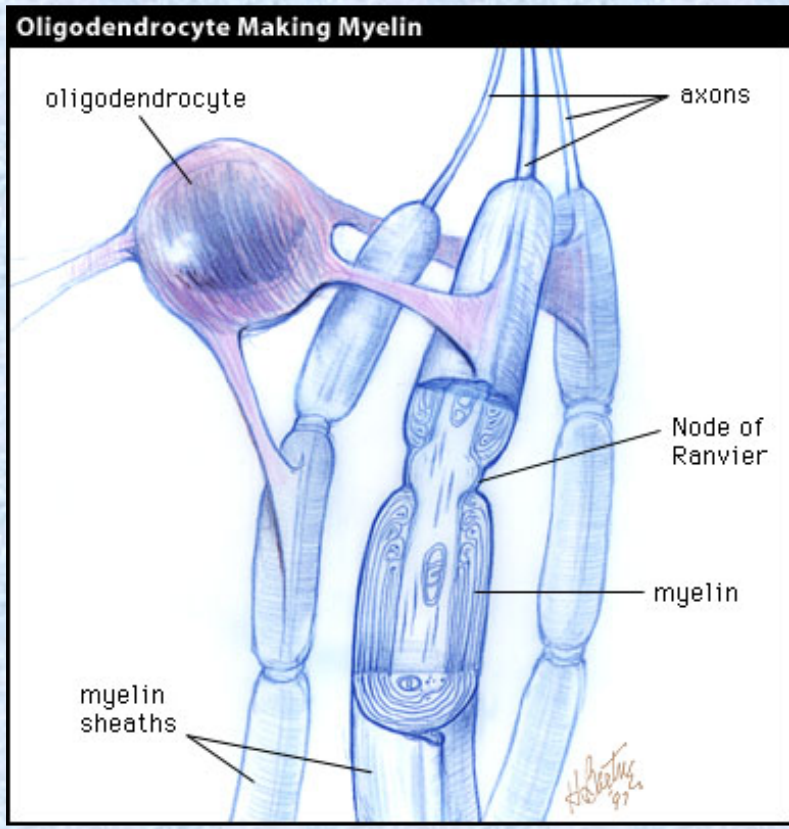


- Are electrical excitable
- Most neurons consist of:
  - A cell body
  - Dendrites for receive input
  - Axons propagate nerve impulses to next neuron
- Action potential propagates along surface of neuron
- Structure and function vary

[4]

CNS Glial Cells

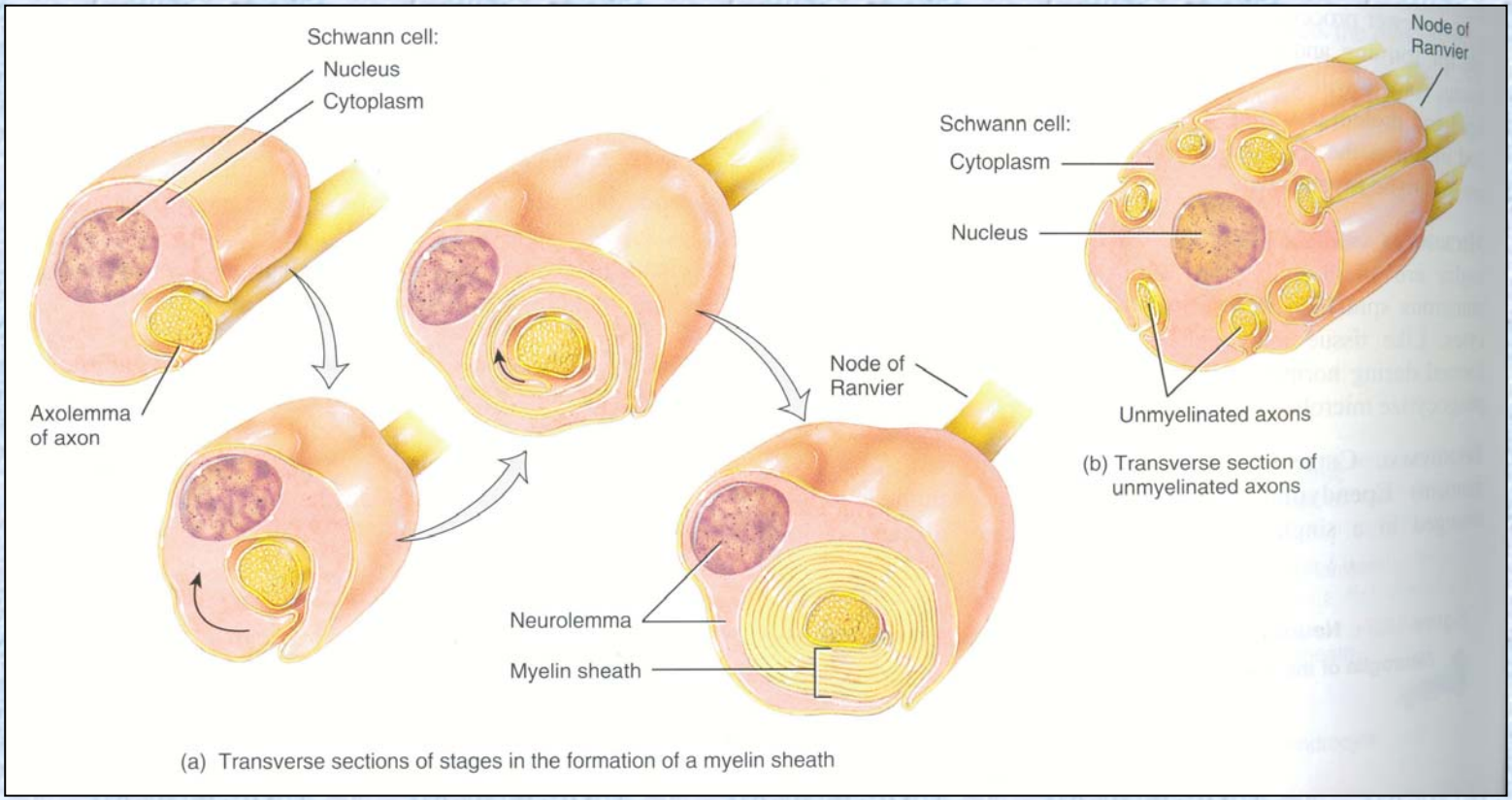
- Several Different Types of Glial cells in CNS
  - **Astrocytes** provide strength, maintain homeostasis
  - **Oligodendrocytes** form a **myelin sheath**
  - **Microglia** remove cellular debris
- The myelin sheath insulates the axon and increases propagation rate **[4]**



[5]

PNS Glial Cells

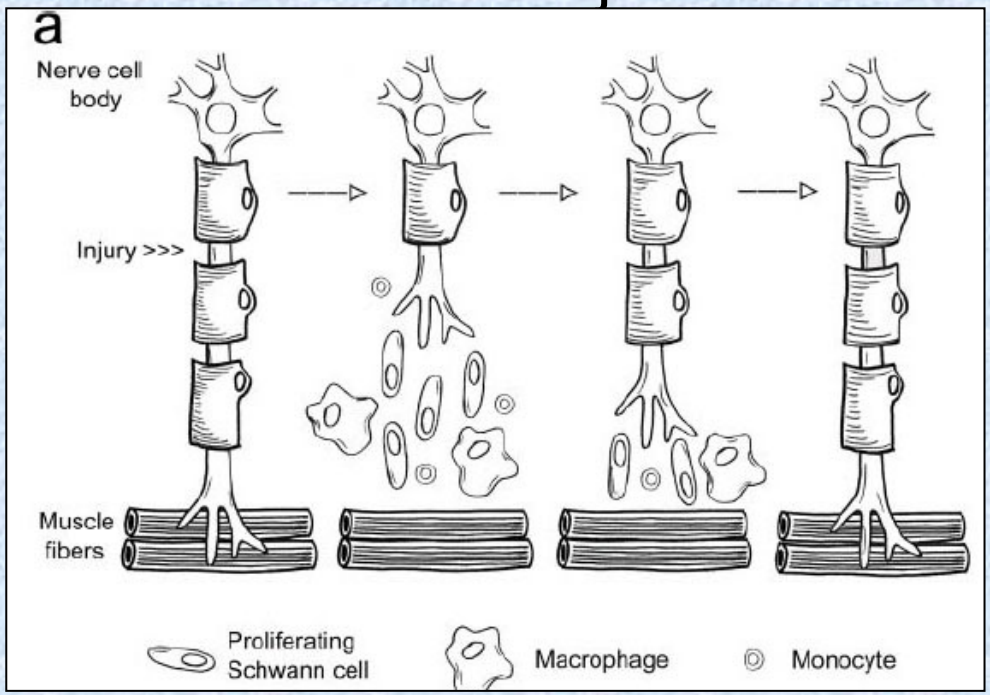
- Different Types of Glia in PNS
  - **Schwann Cells** provide myelin sheath except
    - Insulates only one axon
    - Outer surface (Neurolemma) aids repair



[4]

PNS Nerve Injury

- PNS able to regenerate under limited conditions
  - Gap must be small
  - Nerves ends must be adjacent

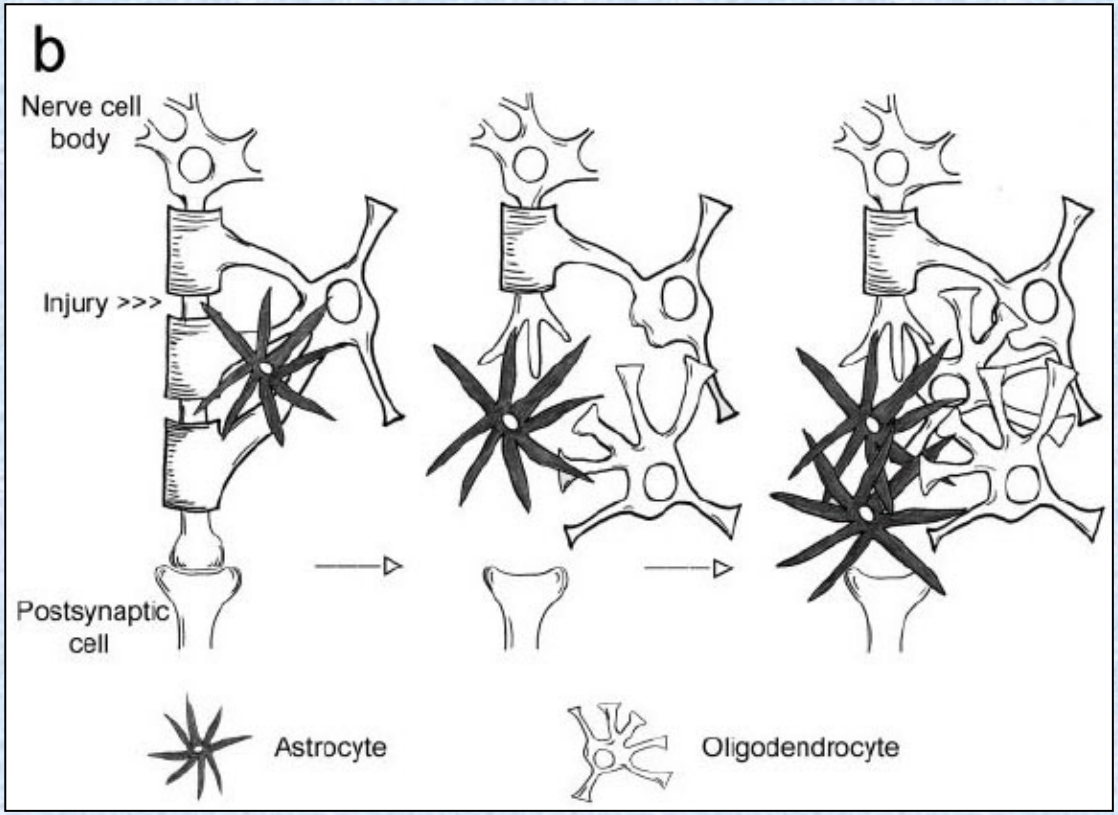


- Variety of methods for repair for more severe injuries

[6]

CNS Nerve Injury

- CNS demonstrates much less ability to regenerate



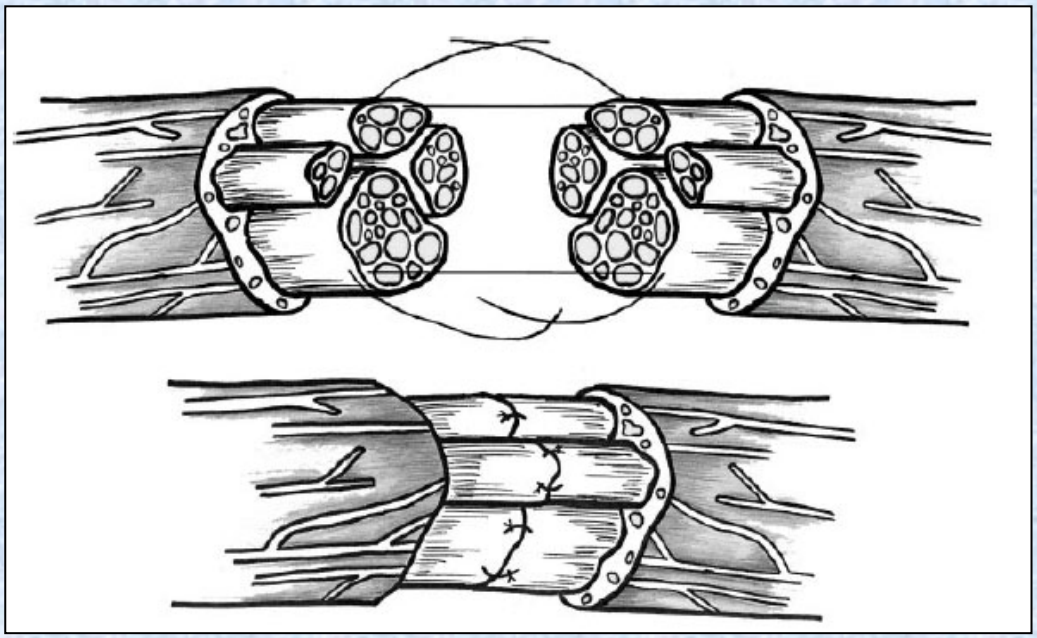
- Few methods for repair, most CNS injury leads to permanent loss of function

[6]

When Things Go Wrong: PNS Nerve Repair

Two Methods for PNS Nerve Repair

1. End-to-end suturing of the nerve
   - Only if no tension
   - Often not possible because ends retract

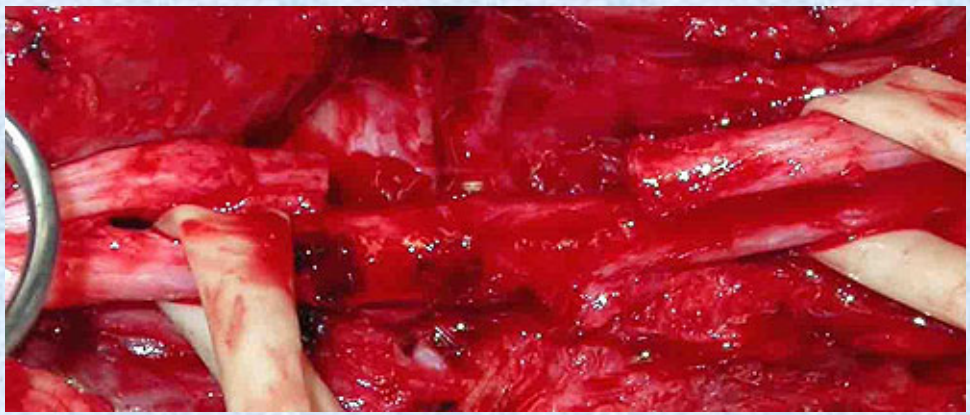


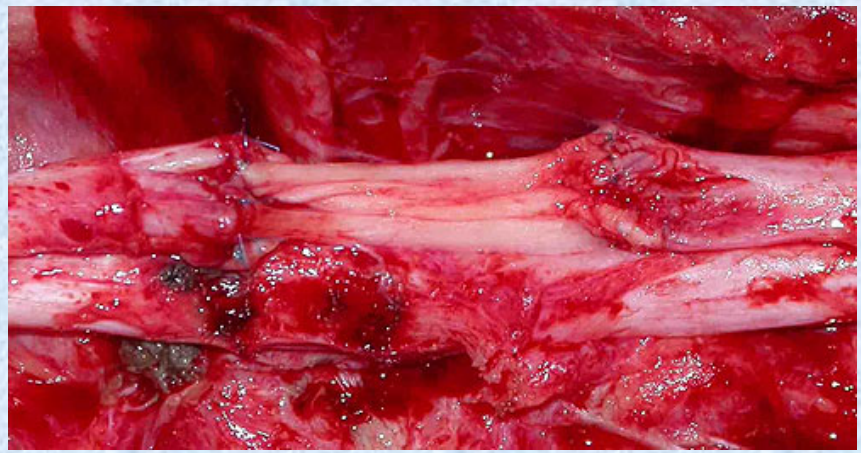
[6]

2. Bridging the gap using
   - Existing nervous tissues
   - Nerve guides

Bridging: Autologous Nerve Grafting

- Currently the best method of nerve repair
- Functional recovery rates approach 80%
- Donor nerve is usually the Sural nerve
  - Up to 40cm **[6]**

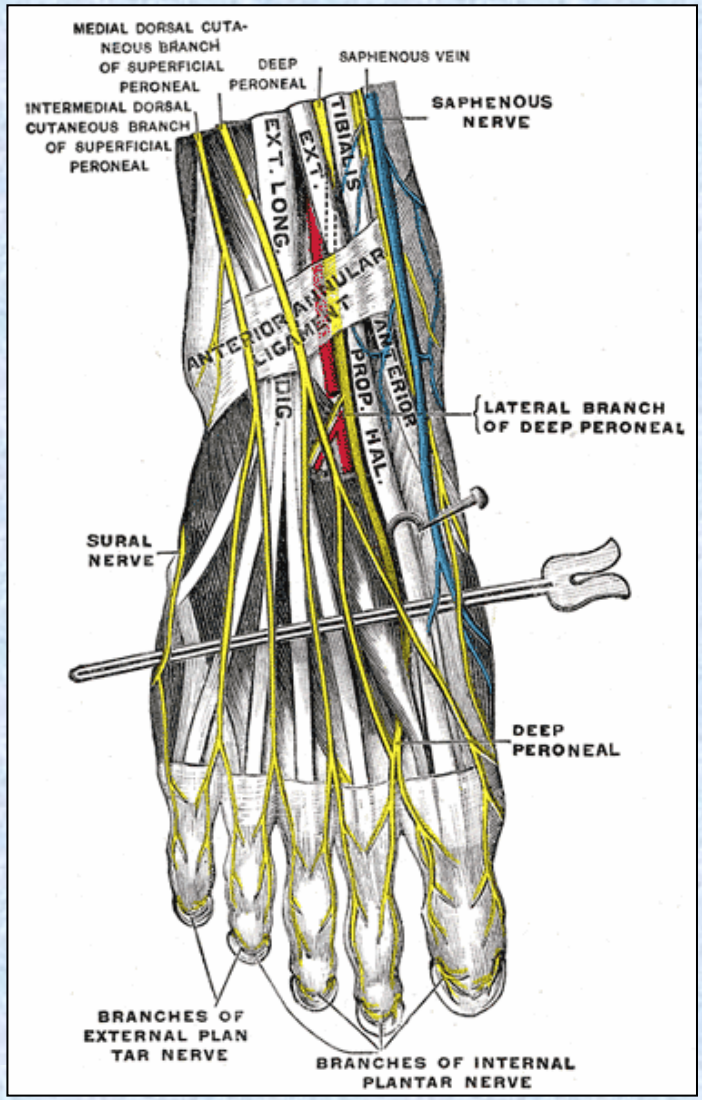




[7]

Autologous Nerve Graft Negatives

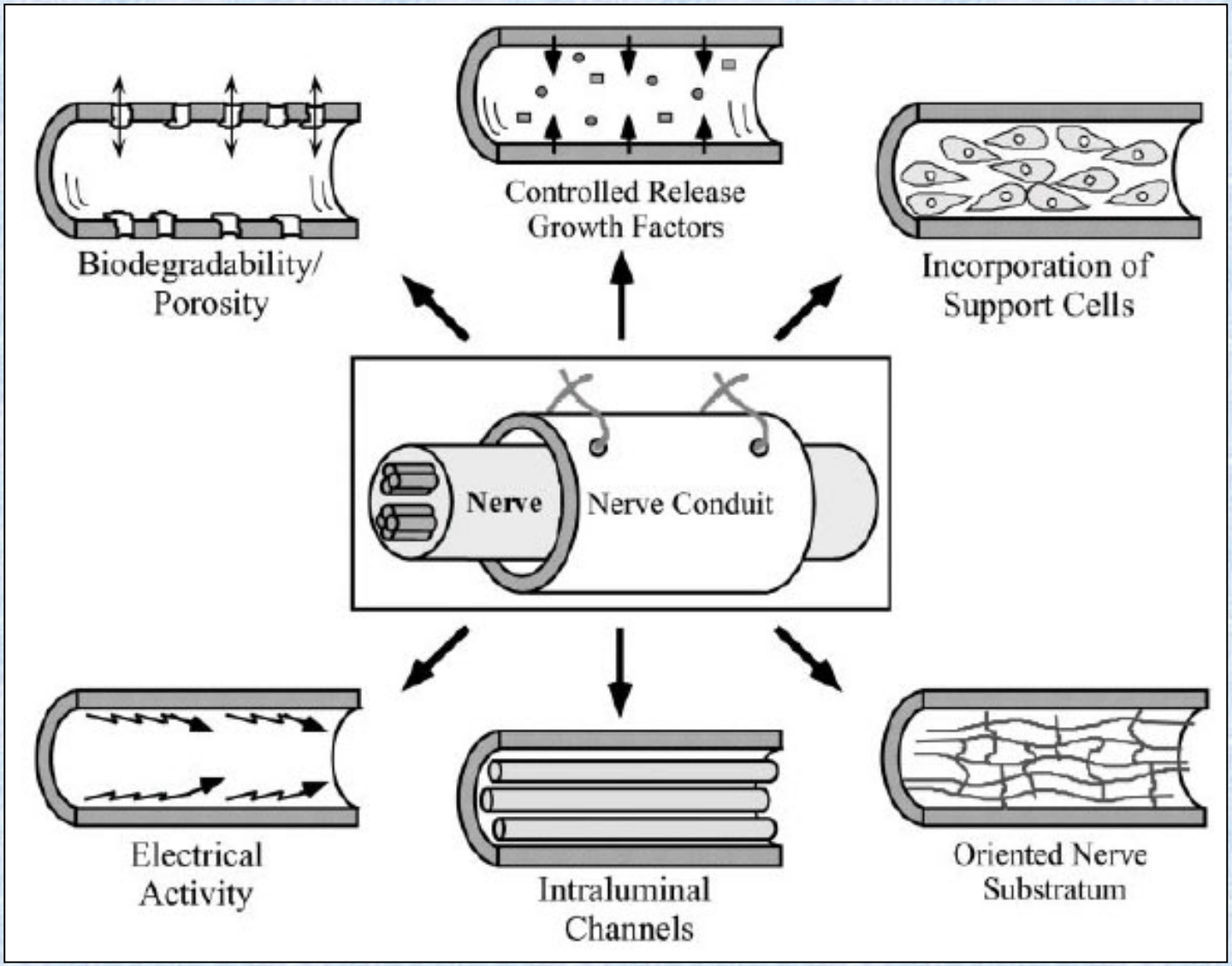
- Donor Site
  - Loss of feeling
  - Neuromas (painful nerve bundles)
- Can require multiple surgeries
- Axon pathways cannot be accurately matched



[8]

Nerve Guides: Building the Better Bridge

Properties of a Desirable Nerve Guide



[6]

Bio-Materials for Nerve Guides (selection)

Non-Biodegradable Synthetic

- Silicone tubes
- SaluBridge®

Biodegradable Synthetic

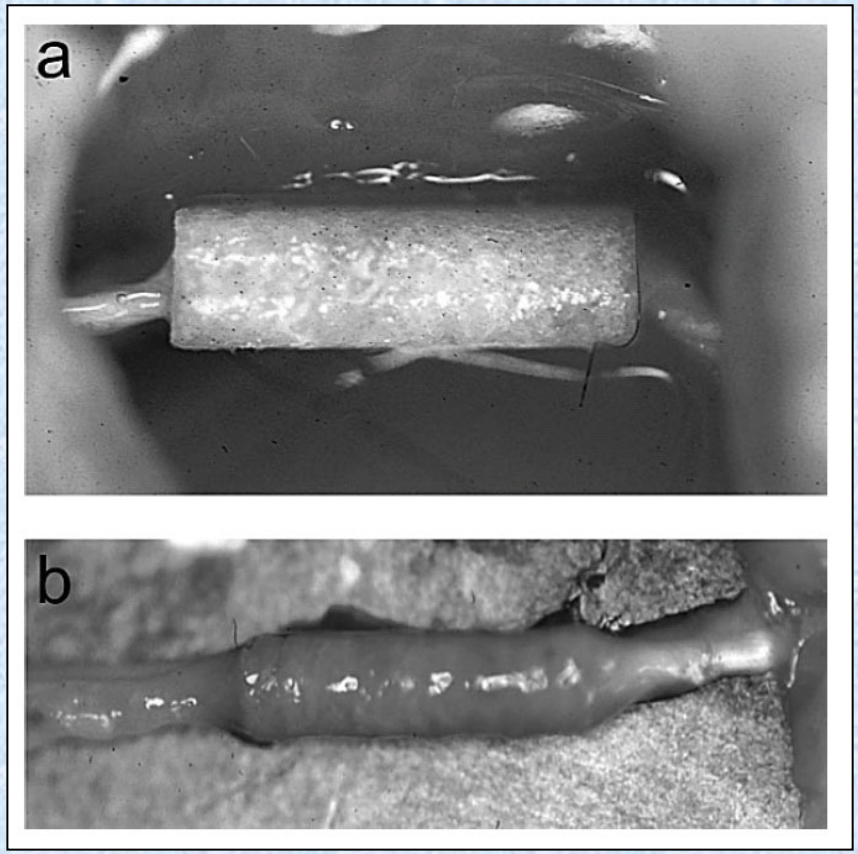
- Poly(esters)

Natural

- Gelatin
- Chitosan
- NeuraGen®

Silicone Tubes

- Currently used in clinical practice
- Up to 5mm
- Provide insulated environment **[2]**



[9]

Silicone Tube VS Direct Suture Study

- In human ulnar nerve, after 1 year
- Small gap size used

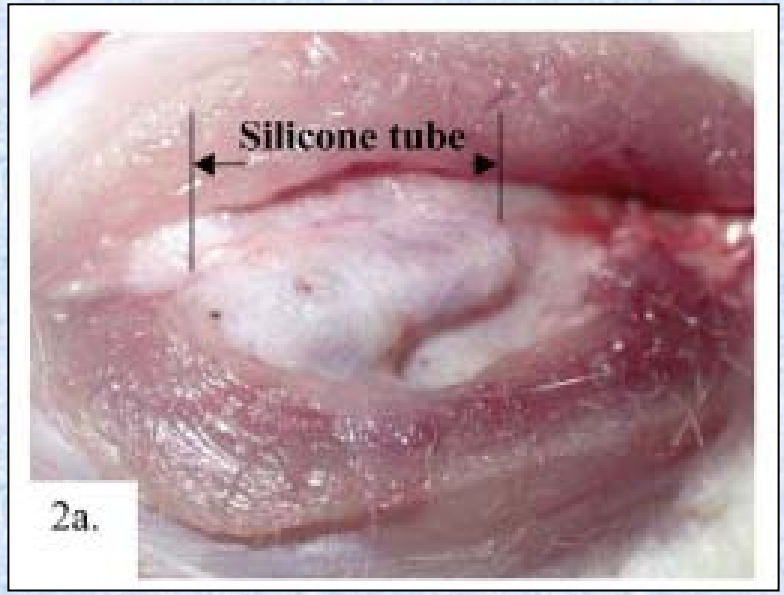
			Conventional repair (n)	*Tubular repair (n)*
S0	No recovery of sensibility in the autonomous zone of the nerve		–	–
S1	Recovery of deep cutaneous pain sensibility in the autonomous zone of the nerve		–	–
S1+	Recovery of superficial pain sensibility		–	–
S2	Recovery of superficial pain and some touch sensibility		3	5
S2+	As in S2 but with overresponse		3	2
S3	s2PD over 15 mm, m2PD over7 mm. Recovery of pain and touch sensibility with disappearance of overresponse	3		5
S3+	s2PD 7 to 15 mm, m2PD 4 to 7 mm.As in S3 but good localization of the stimulus and imperfect recovery of two-point discrimination		3	3
S4	s2PD 2 to 6 mm, m2PD 2 to 3 mm. Complete recovery	0		1

[7]

Silicone Tube Negatives

- Doesn't promote cell adhesion
- Not biodegradable
- Not permeable
- Fibrous encapsulation
  - chronic inflammation
  - chronic compression

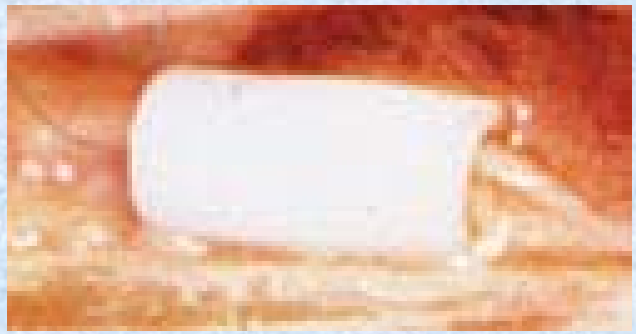
Silicone tubing was removed in 8 of 17 cases



[7, 9]

SaluBridge Nerve Cuff

- Currently in clinical use
- FDA and Canadian Medical device licenses
- Constructed of biogel material
- Fully biocompatible, but not biodegradable
- Semi permeable
- Can repair up to 5 cm
- Provides improved results over silicone* **[9]**

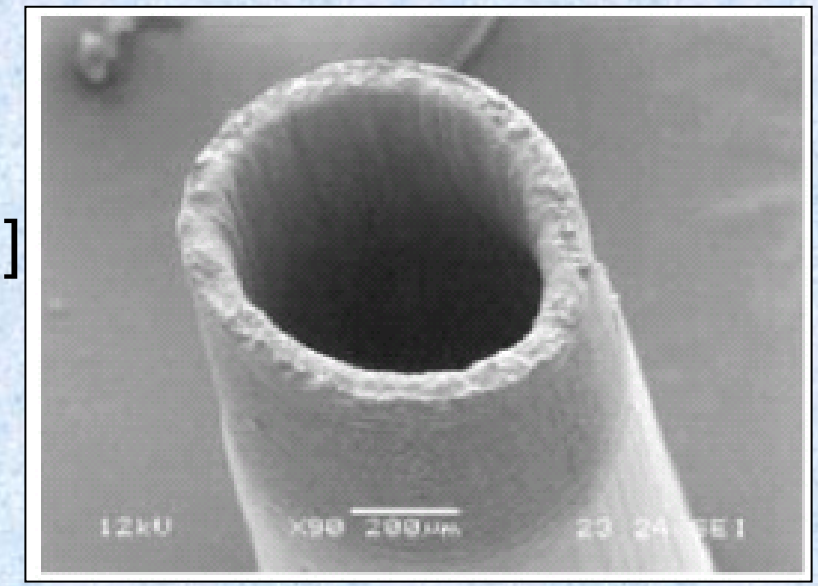


Synthetic Biodegradable Nerve Guides

Poly(ester) Based Nerve Guides

Includes Poly-(caprolactone) (PCL), Poly-(L-latic acid) (PLLA), Poly(latic-co-glycolic acid) (PLGA)

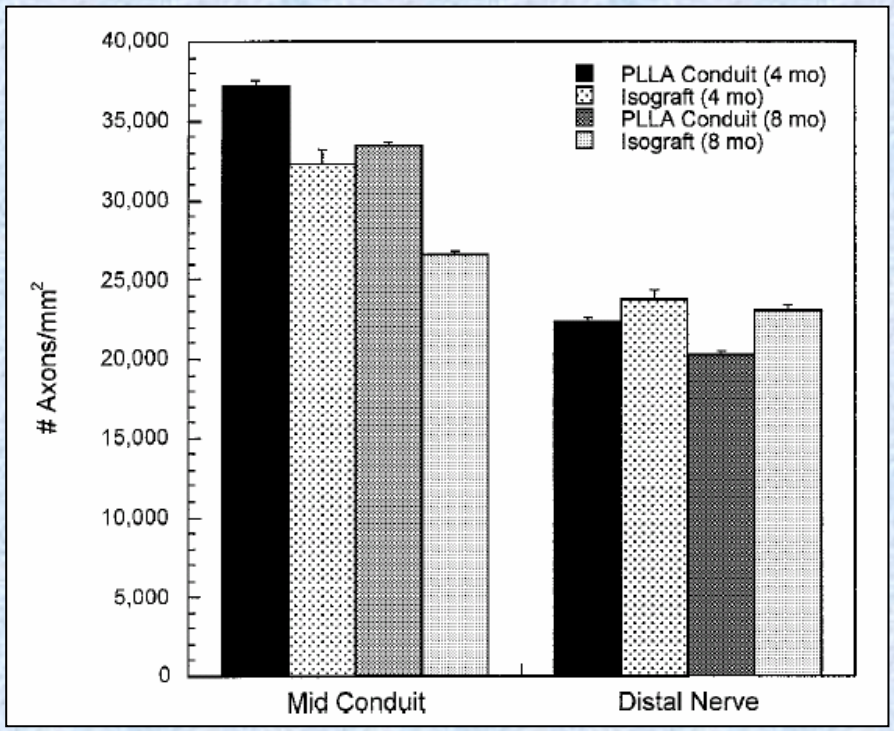
- Biodegradable
- Porous to allow vascularization
- Increased biocompatibility
  - Avoids inflammation and pressure
- Flexible: properties can be engineered
- Promotes cell adhesion
- Currently in animal trials
- Materials approved by FDA [2,3,6]

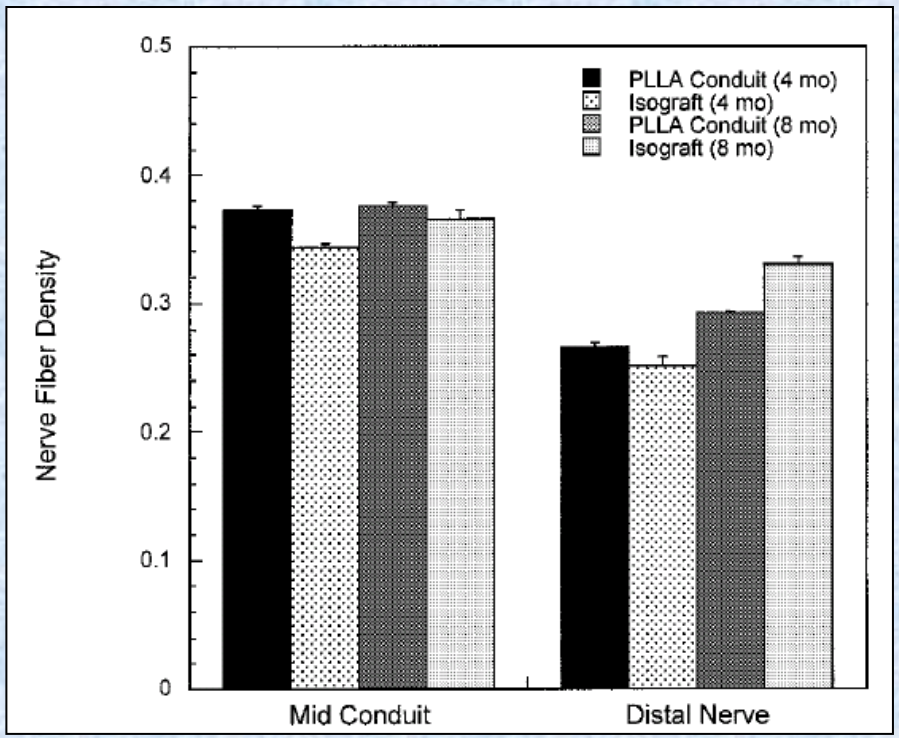


[2]

PLLA vs. Isograft Study Results

- In rat sciatic nerve model n=21





[10]

Natural Biodegradable Nerve Guides

Natural Polymer Nerve Guides

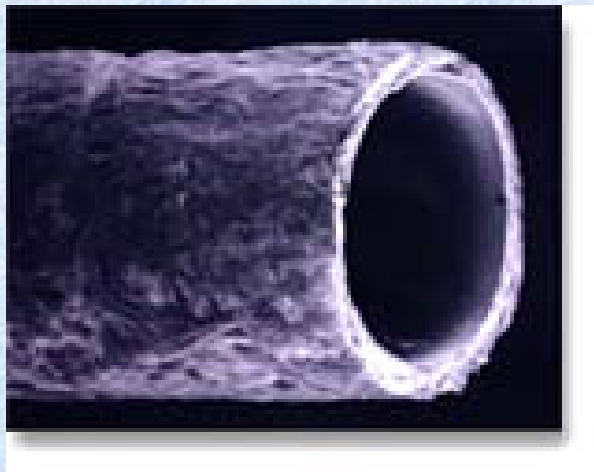
- ECM based guides
  - Laminin,Fibronectin, Collagen/Gelatin
  - Biodegradable, resorbable
  - Excellent biocompatibility
  - Excellent porosity
- Chitosan
  - Derived from Chitin
  - Natural tubes can be obtained from Crab tendons
  - Currently in research phase [2]

[11]

[12]

NeuraGen® Nerve Guides

- Collagen based nerve guide
- Up to 3 cm
- Semi-permeable
- Biocompatible, resorbable
- Level of functional recovery equal to direct suture*



[13]

Enhancing the Functionality of Nerve Guides: Building the Better, Better Bridge

Methods for Improving Nerve Graft

- Impregnating Schwann Cells
- Filling Guide with ECM material liquid
- Filling Guide with ECM scaffold from donor
- Filling Guide with ECM material (magnetically aligned)
- Laser sintering a structure into a Poly(ester)
- Poly(ester) based foam

Common property is introducing structure into the guide

[2,3,6]

Saline vs. Collagen vs. Magnetically Aligned Collagen

- Used in NeuraGen Nerve Guides, mouse sciatic nerve

Definition of Study Groups

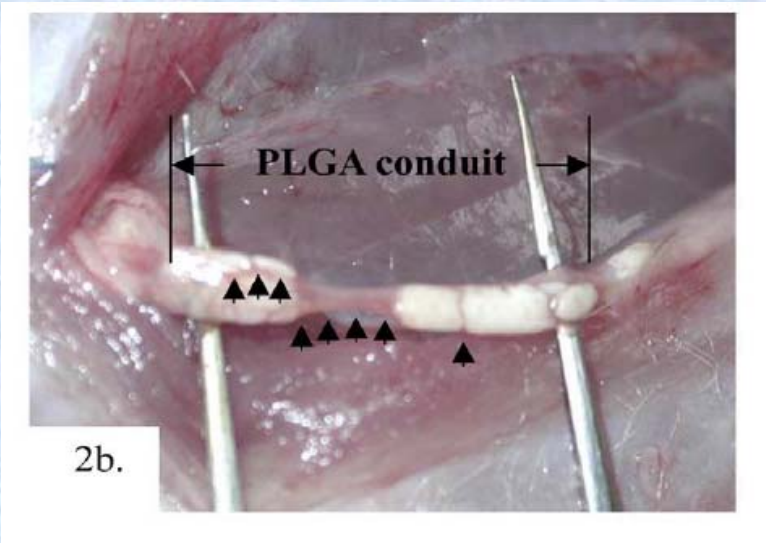
Groups	*n*	Gap (mm)	Tube content
Unoperated	9	—	—
S4	3	4	Saline solution
C4	11	4	Control collagen gel
C6	11	6	Control collagen gel
M4	6	4	Magnetically aligned collagen gel
M6	8	6	Magnetically aligned collagen gel

Proportions of Mice with Regenerated Nerves, as Judged by the Presence of Myelinated Nerve Fibers Regenerated at Midtube and Distal to the Tube after 30 and 60 dpo

	No. reg/total at 30 dpo		No. reg/total at 60 dpo	
Group	Mid	Distal	Mid	Distal
S4	1/3	0/3	—	—
C4	3/5	3/5	3/6	2/6[b]
C6	0/5[a,c]	0/5[c]	3/6	1/6[a,b]
M4	3/4	0/4	4/4	3/3[d]
M6	0/4[a]	0/4	4/4	4/4

[14]

Using Low Intensity Ultrasound (US) with Nerve Guides



The regenerated nerve

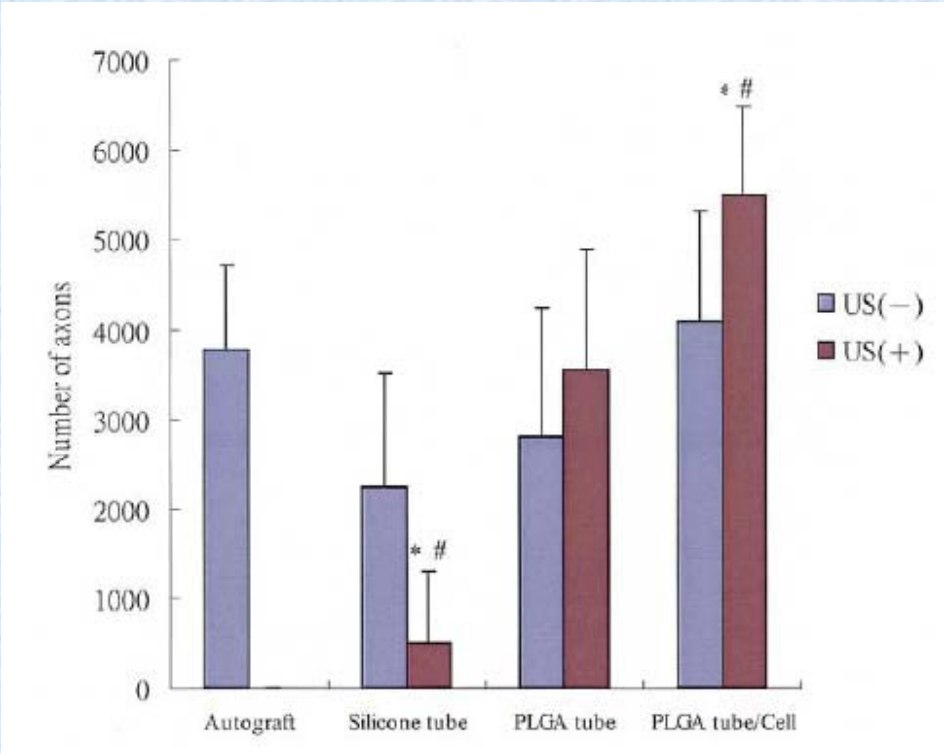
6 weeks

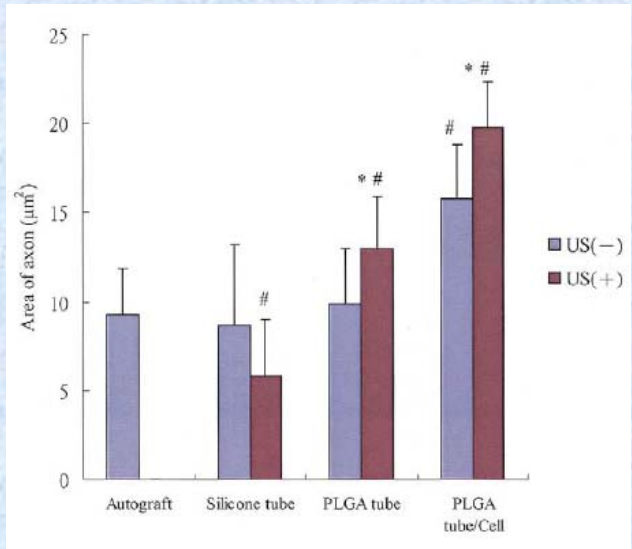
- Low Intensity: 0.2 W/cm^2
- Proven beneficial effects on soft tissues
- Applied in conjunction with nerve guides seeded with Schwann cells to enhance cell functions.
- Tested while bridging 1.0 cm gaps on rats with PLGA **poly(DL-lactic acid-co-glycolic acid)** nerve guides seeded with Schwann cells

[A]

Results of Low Intensity US Stimulation

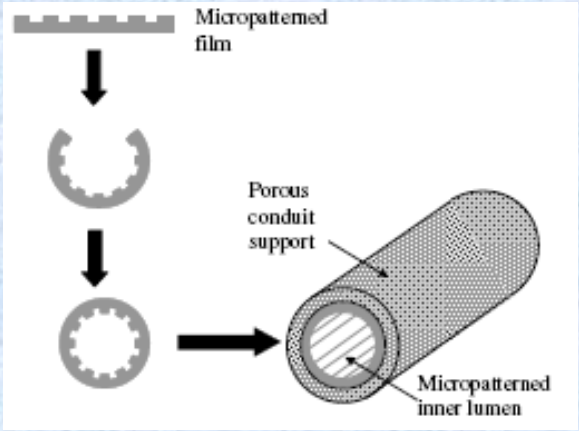
- After 6 weeks autografting resulted in 3800 regenerated axons.
- PLGA with Schwann cells and Ultrasound: 5500
- Currently under additional experimentation in rats to obtain a full animal model.

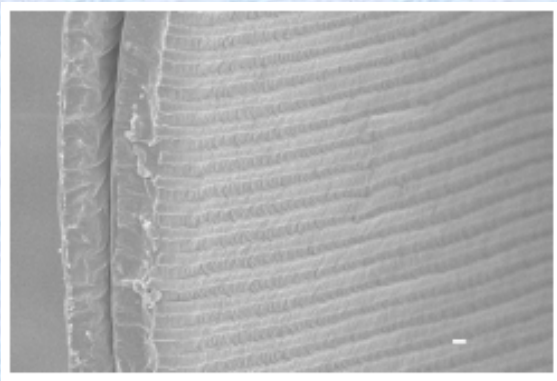




[15]

Micropatterning the inside of Nerve Conduits



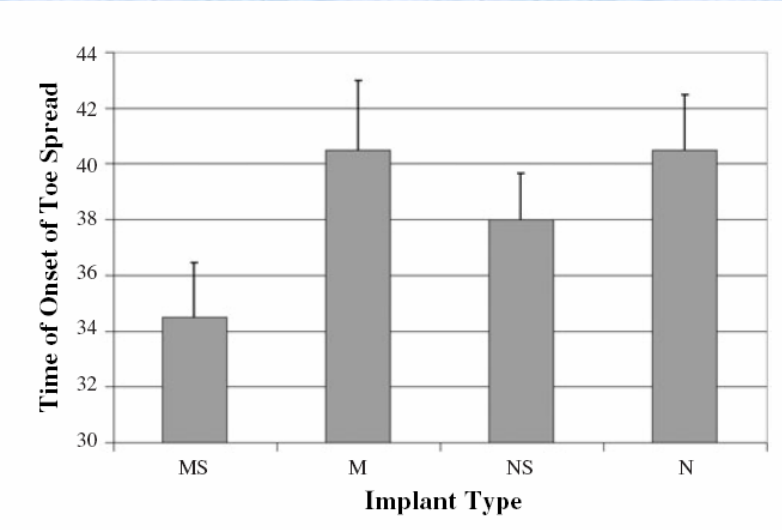


Width: 10 um Depth: 4.3 um Distance Between: 10 um Thickness of film: 40 um

- Micropattern is created on a silicon/quartz wafer.
- PDLLA (poly(D,L-lactic acid)) is molded on the wafer
- Dimensions of film : Determined through in vitro experiments
- Films are impregnated Schwann cells, then inserted into nerve guides.

[16]

Effects of Micropatterning

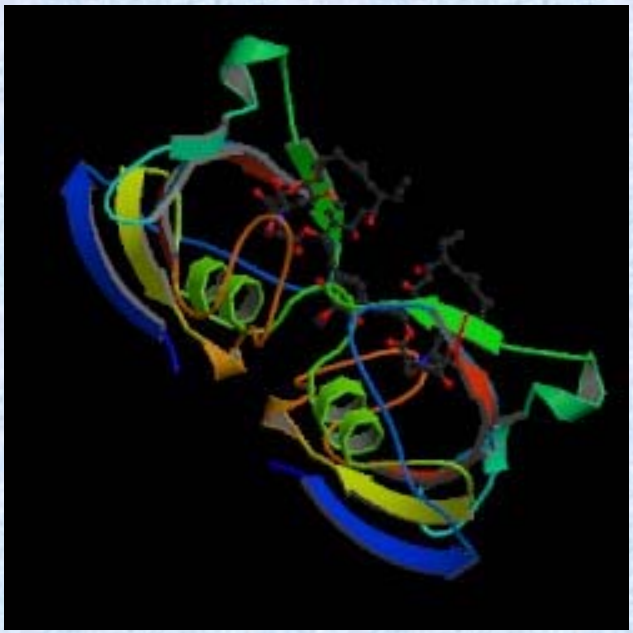


- Using a micropatterned inner surface in conjunction with a Schwann cell impregnated nerve guide doubles the rate of functional recovery.
- The Micropatterned inside the nerve guide enhanced Schwann cell activity.
- Using micropatterned nerve conduits cut recovery time in half in rat test subjects
- Increase in overall axonal growth.

- Currently undergoing long term Animal trials by Octillion Corp. [17]

[16]

Immunosuppressant Neurotrophins



FK506 - Structure **[19]**

- Neurotrophins: Enhance Nerve Growth: NGF, NT-3, NT-4
- CsA (Cyclosporin A), Rapamycin, FK506 (Tacrolimus)
- Highly effective at preventing graft rejection.
- FK506 can double the number of axons that regenerate following a nerve injury and increase Myelination by up to 40%
- Daily injections of CsA or FK506 can induce regeneration of axons in the spine.
- Can be taken orally.
- Available as a general immunosuppressants **[18]**

CNS: Building the Better Brain

Barriers to Regenerating the CNS

- Secondary Damage to nerves:
  - Final nervous tissue damage surpasses initial damage.
  - Additional damage caused cells being deprived of nutrients
- Physical Barriers:
  - Gaps in nervous tissue caused by phagocytosis of dead cells
  - Formation of Scar tissue that axons can't penetrate
- Biological Factors:
  - CNS Myelin and Myelin associated proteins such as Nogo-A have been identified as growth inhibitors since 1988

[20]

CNS Treatments

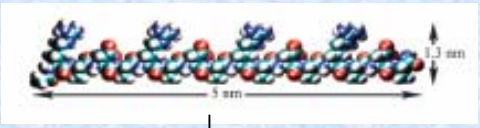
[21]

- Preventing Secondary cell damage: most attempts unsuccessful.
- Suppression of growth inhibitors using antibodies
- Enhancing growth response using Neurotrophins delivered via:
  - Injections
  - Grafts of genetically modified cells
  - Viral Delivery systems
- Rehabilitation: Physiotherapy and Ergotherapy - only established treatments for human spinal chord injuries
  - Few standardized treatment methods, or models of operation

[20]

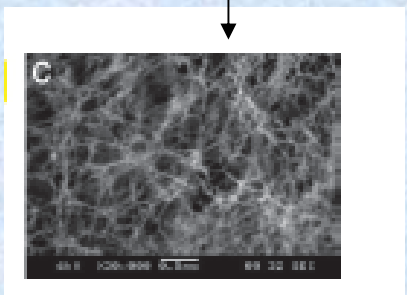
Using Nanofiber Scaffolds to Repair Brain Damage

Monomer Molecule for SAPNS



SAPNS Solution is injected into damaged region of the CNS

SAPNS combines with Ionic Fluid (Cerebrospinal Fluid)



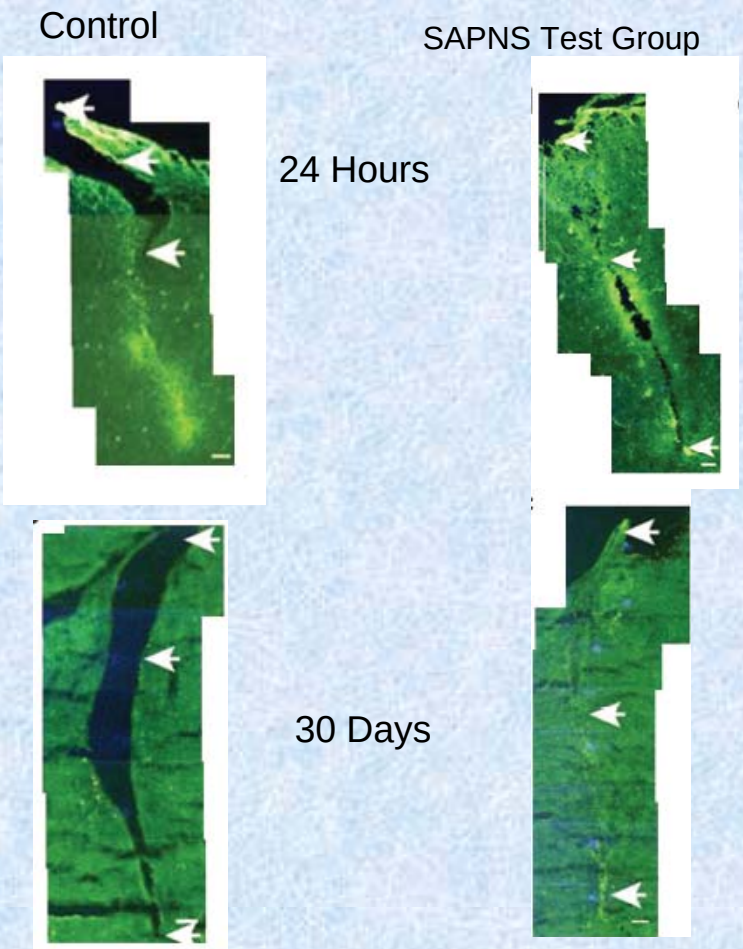
Scaffold Network

- Self Assembling peptide nanofiber Scaffolds (SAPNS):
- Provides a framework for axonal growth
- Inhibits scar formation.
- The scaffold is at the same scale as the extracellular matrix
  - Direct interaction is possible
- Scaffold can be absorbed by nerve cells over time.
- Non-toxic, and Immunologically inert
- Has just finished initial animal testing (Hamsters)

[22]

Nerve Regeneration after Transection of the Optic Tract

Perisagital Sections of the Optic Tract

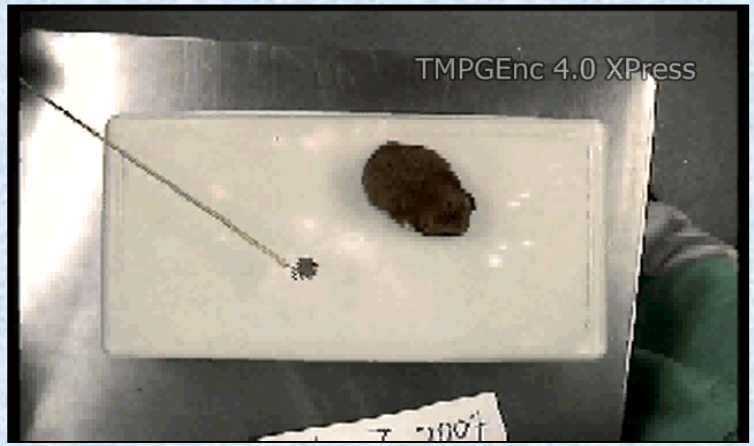


- Most recent experiment: Deep Transection of Optic tract in hamster midbrain.
- SAPNS Treated Cases: gap began closing in the first 24h after surgery. Over time – was reduced or eliminated.
- No progress made in control cases. Scar tissue formation made regeneration of axons impossible

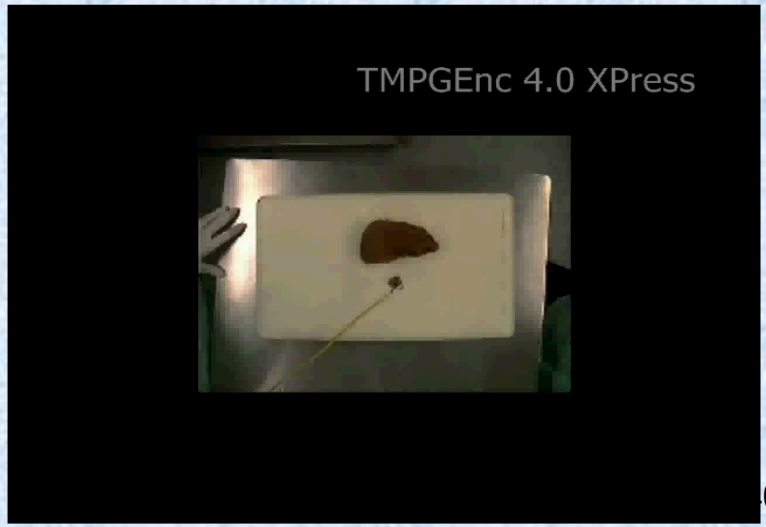
[22]

Results of SAPNS Treatment

- 92% of SAPNS treated hamsters showed axonal growth.
- Nerve density in treated hamsters regenerated on average to 78% of normal.
- **Visual ability returned to 75% of treated animals.**
- Treated animals turning responses were ~ 29% slower than normal



Control Hamster: Blind



SAPNS Hamster: Sighted

[22]

Conclusion

- There are numerous biomaterials both in current use, and under development for treating nerve lesions
- Of all current clinical procedures autografts remain the most successful
- Emerging Nerve Guide materials, and associated treatment techniques are beginning show better experimental results than traditional autographs.
  - Low Intensity Ultrasonic Stimulation with Schwann Cell impregnated PLGA Nerve Guides
- Technology is emerging that can induce regeneration of damage to CNS axons.
  - SAPNS
- Experimental biomaterials and methods are being optimized
- The Future Looks Bright!

Thanks!

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