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Channel density and porosity of degradable bridging scaffolds on axon growth after spinal injury

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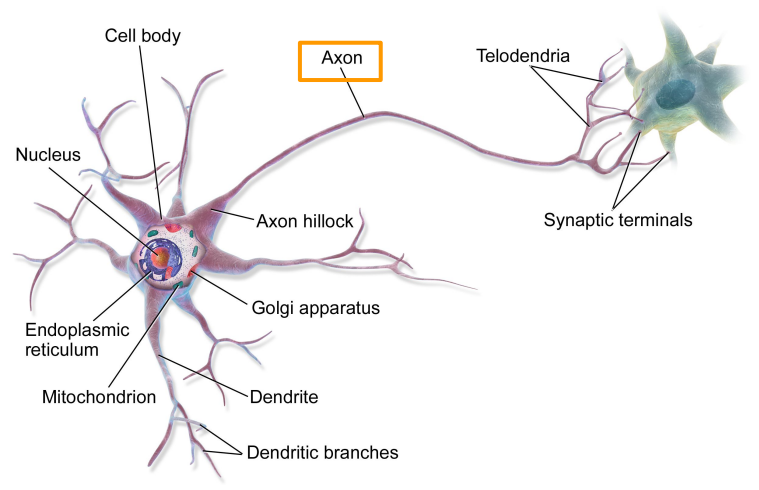
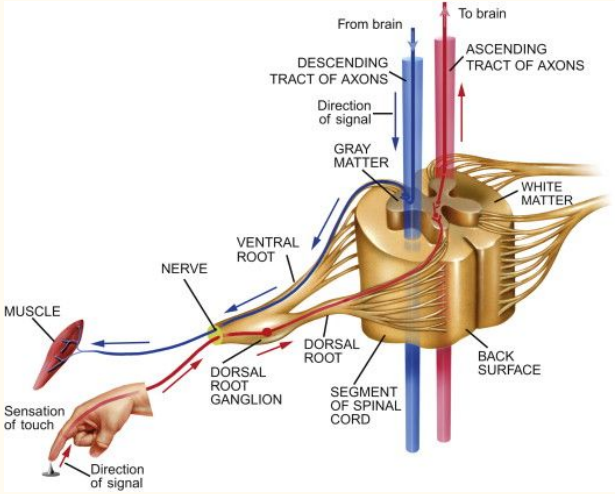
Introduction

- The problem: no spinal cord regeneration post-injury
- Potential solution: material (the bridge) that directs axon extension and enhances recovery
- We are looking at how changes in bridge architecture improve axon extension



https://en.wikipedia.org/wiki/Axon



https://www.sciencedirect.com/science/article/pii/B9780123983589000641

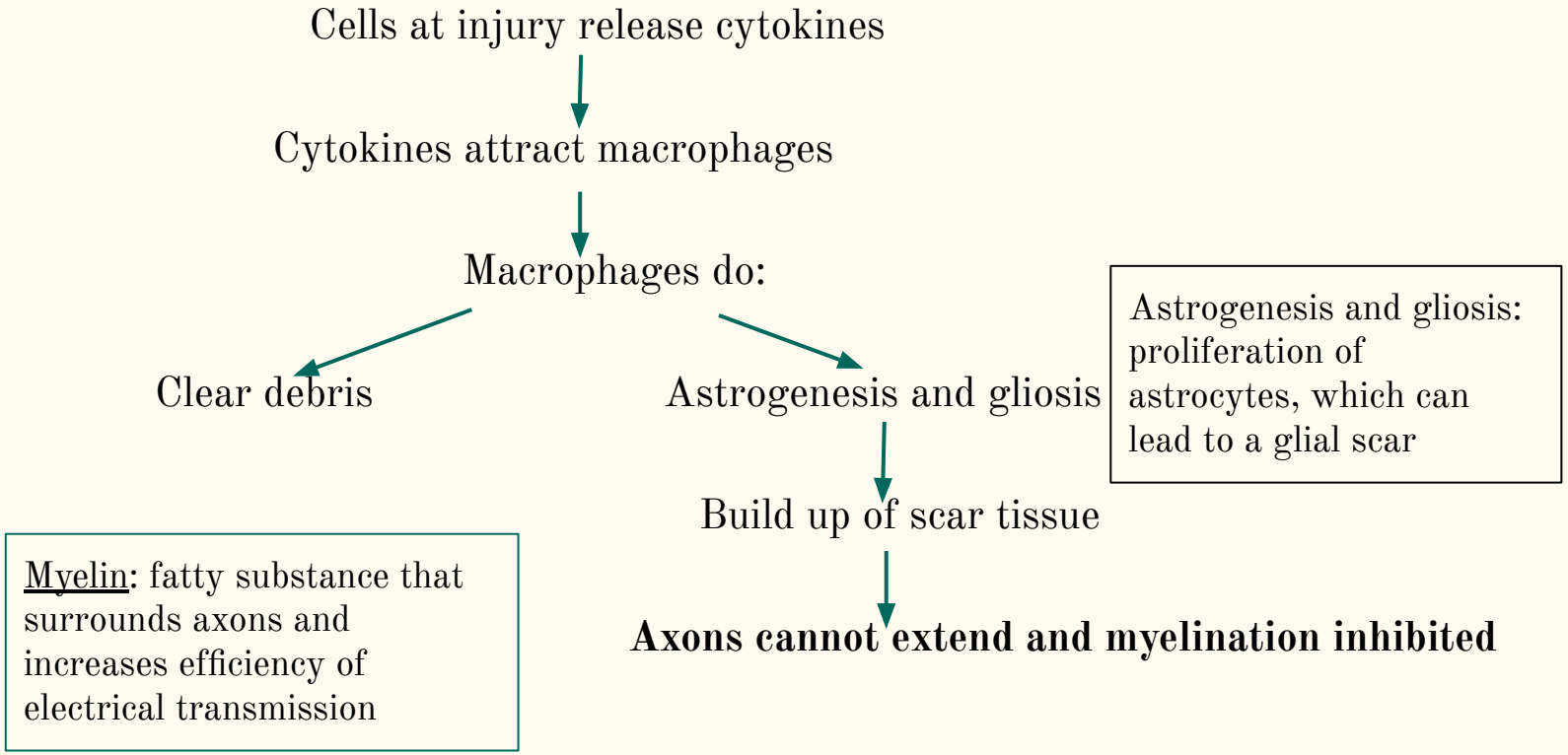
Outline

1. Background information
2. Bridge Structure
3. Cellular Residency
4. Myelination
5. Axon elongation
6. Extracellular proteins
7. Conclusion
8. Limitations/new research
9. Q&A

Feel free to ask any questions throughout the presentation!

Background Info

What happens after spinal cord injury?



What do we *want* to happen after injury?

We want:

- mature oligodendrocytes that myelinate
- axons to extend across the injury

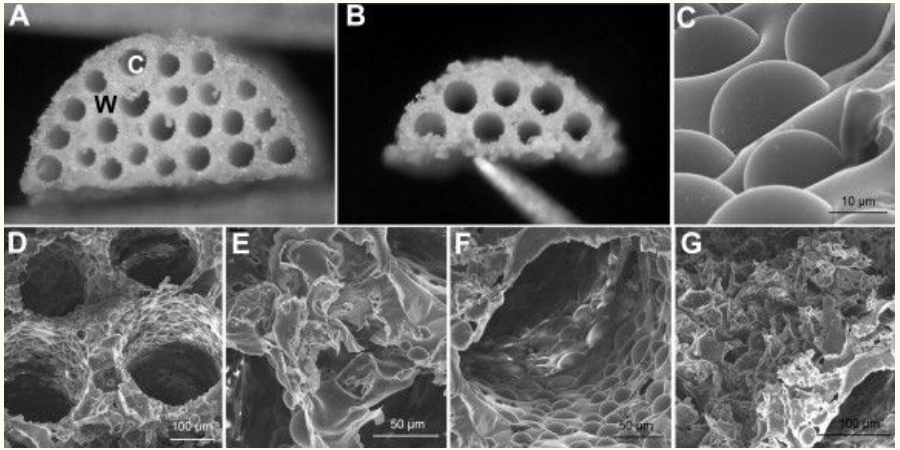
Oligodendrocytes: produce myelin in the central nervous system

Myelin: fatty substance that surrounds axons and increases efficiency of electrical transmission

How can this be accomplished?

Bridges!

- Bridges help guide the axons so they grow in the right direction
- Reduces number of inflammatory cells
- Enhances functional recovery



Previous experiments and the creation of bridges

knowledge from previous experiments

- Bridges with channels facilitate axon extension
- Solvent casting can be used to create bridges
- Gas foaming technique can be used to create porous structures

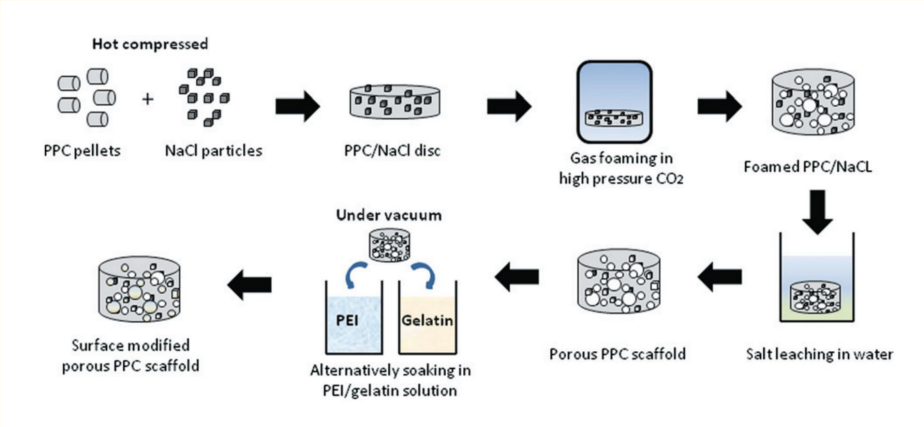
This experiment included:

- Sucrose fiber template
  - Greatly increases density of channels
  - gives greater control over porosity

Bridge Structure

Bridge Creation

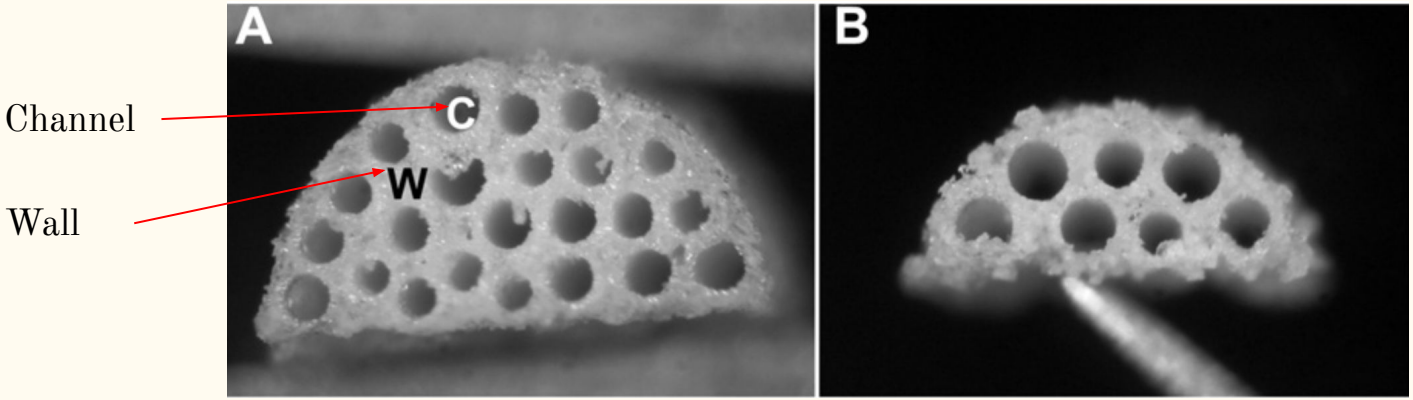
- Goals: high channel density and porous enough to allow neurite connections
- Gas-Foaming procedure
  - Mixed sucrose fibers with PLG microspheres and salt crystals, then packed into a mold
  - The polymeric material is combined with a gas at high pressure
  - Gas molecules at high pressure saturate the polymer, then the temperature is lowered
  - Molecules cluster to form pores, which grow as the gas diffuses



https://doi.org/10.1039/C2GC35608B

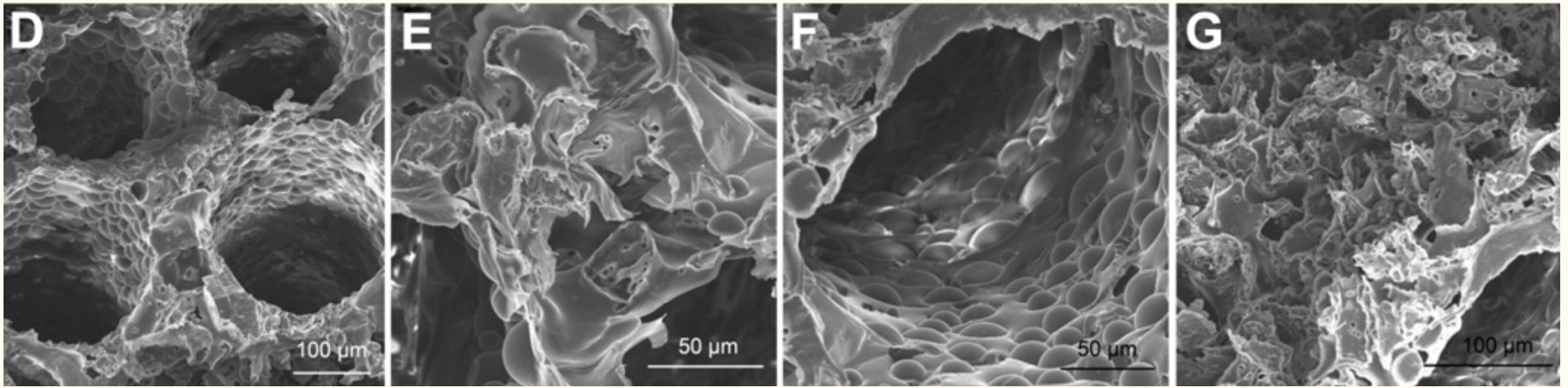
Bridge Creation

- Gas-foaming technique is not consistent on its own
- Particulate-leaching method
  - Bioscaffold is dunked in water to dissolve the porogens, salt and sugar
  - Creates more pores and enhances their interconnectivity
- Result: a porous bridge with multiple channels able to support tissue growth



Specifications

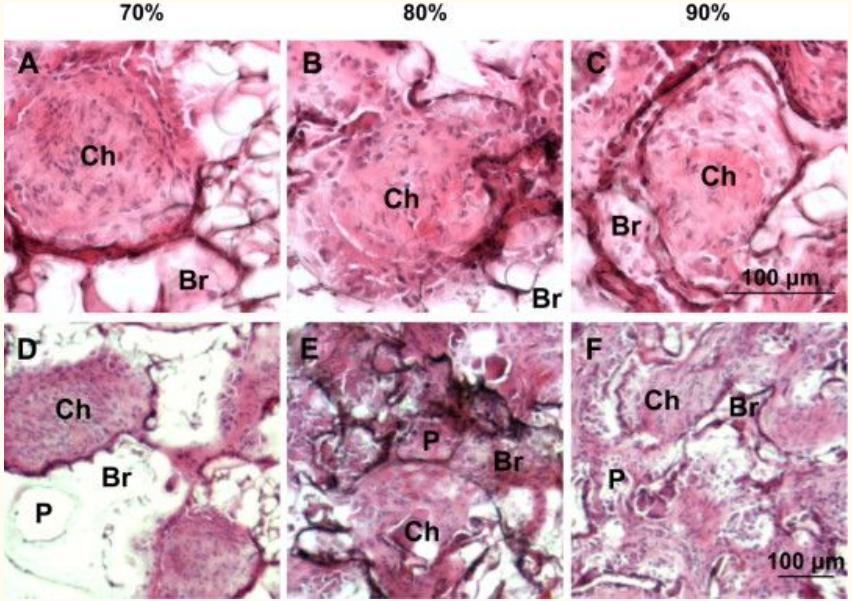
- Rat model: 3.8 * 2.5 * 1.5 mm with 22 channels
- Mouse model: 2.25 * 1.25 * 0.75 mm with 7 channels
- Porosities tested: 70%, 80%, 90%
  - With salt and PLG microspheres
- Average channel diameter: 234 ± 18 μm



Cellular Residency

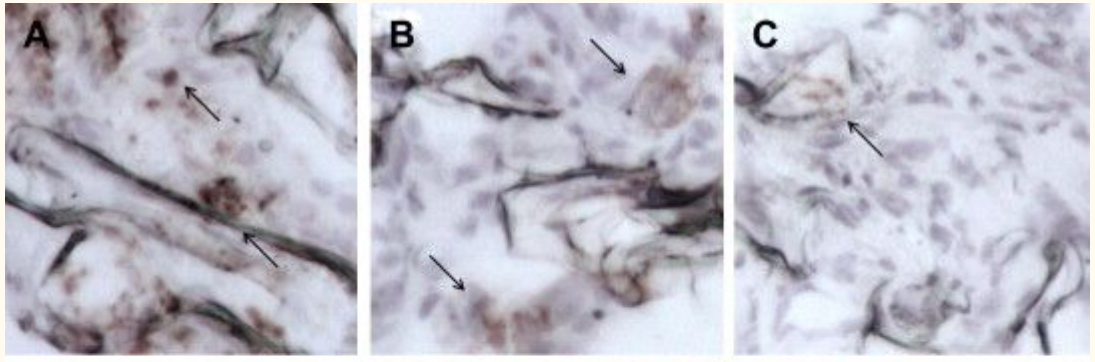
Cellular Residency

- Examining cell infiltration (distribution & identity)
- Goal for the cells: create environment for axon growth
- Figure
  - A-C: channels intact
  - D-F: show infiltration
- **↑ porosity → ↑ infiltration**



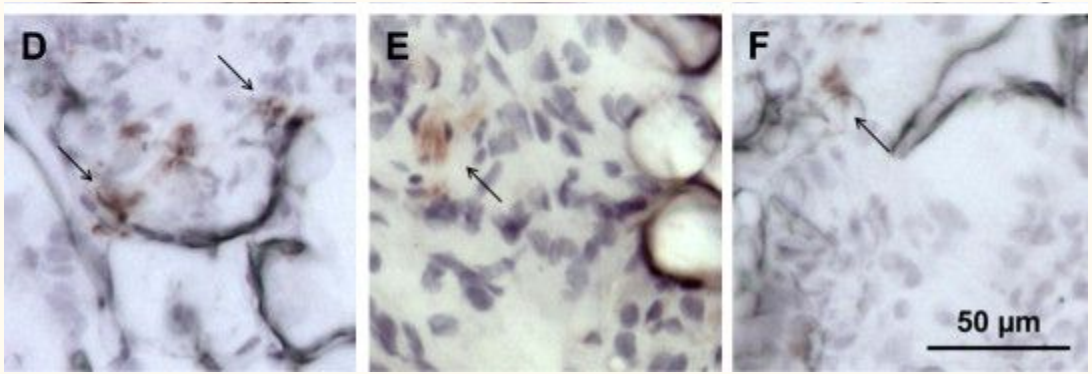
Cellular Residency: Cell Types

- Types associated with injured spinal cord:
  - Macrophages (ED-1+)
    - ↑ with porosity
  - Fibroblasts (rPH+)
    - Not vary
  - Astrocytes (GFAP)
    - Not vary



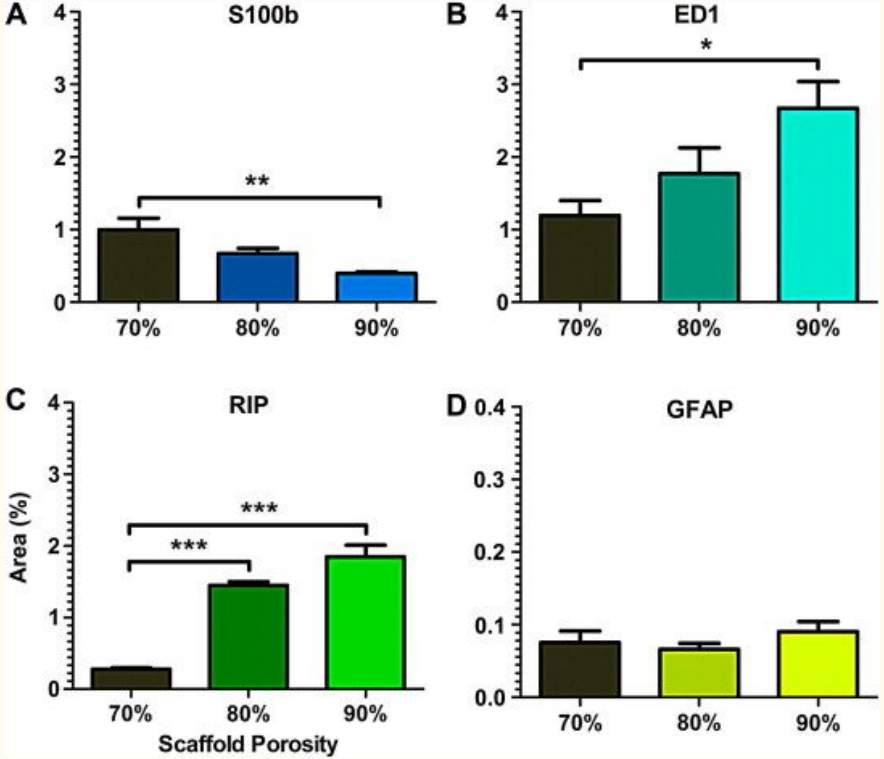
Cellular Residency: Cell Types

- Types associated with regenerating spinal cord
  - Oligodendrocytes (RIP+)
    - ↑ with porosity
  - Schwann Cells (S100β)
    - ↓ with porosity
  - Endothelial Cells (RECA-1+)
    - Not vary



Cell Residency: Conclusion

- Increase in cell infiltration overall!
  - Governed by architecture (porosity)
- Cell types varied based on porosity



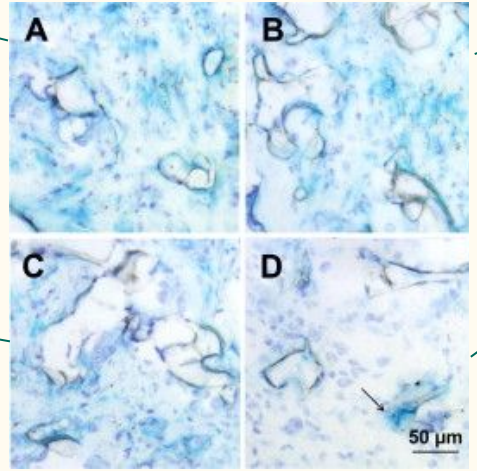
Myelination

Myelination identification

Myelin identified using Luxol Fast Blue Staining, data taken at 2 and 8 weeks

A) 70% porous (8 weeks)

B) 80% porous (8 weeks)



C) 90% porous (8 weeks)

D) (2 weeks)
- Found myelin on periphery of bridge
- Most likely macrophages carrying debris from injury

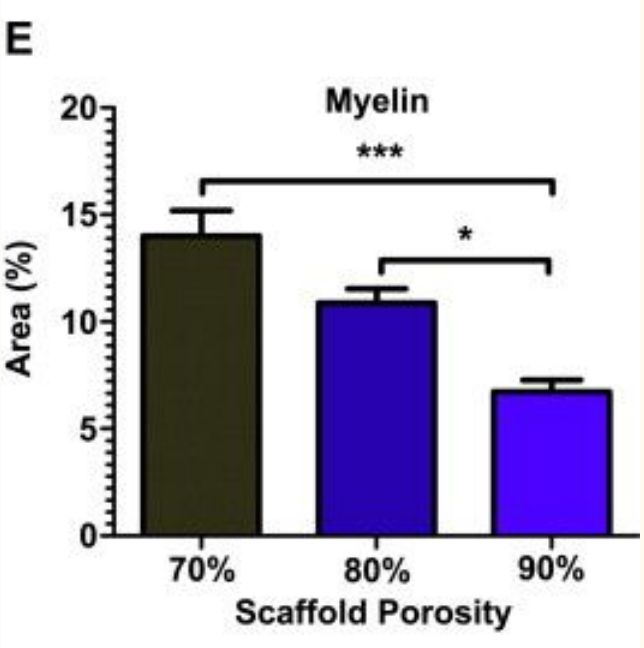
8 week trials saw myelin in the middle of the bridge. Suggests myelin not debris

Porosity Effect on Myelin Production

The amount of myelin varied inversely with the porosity

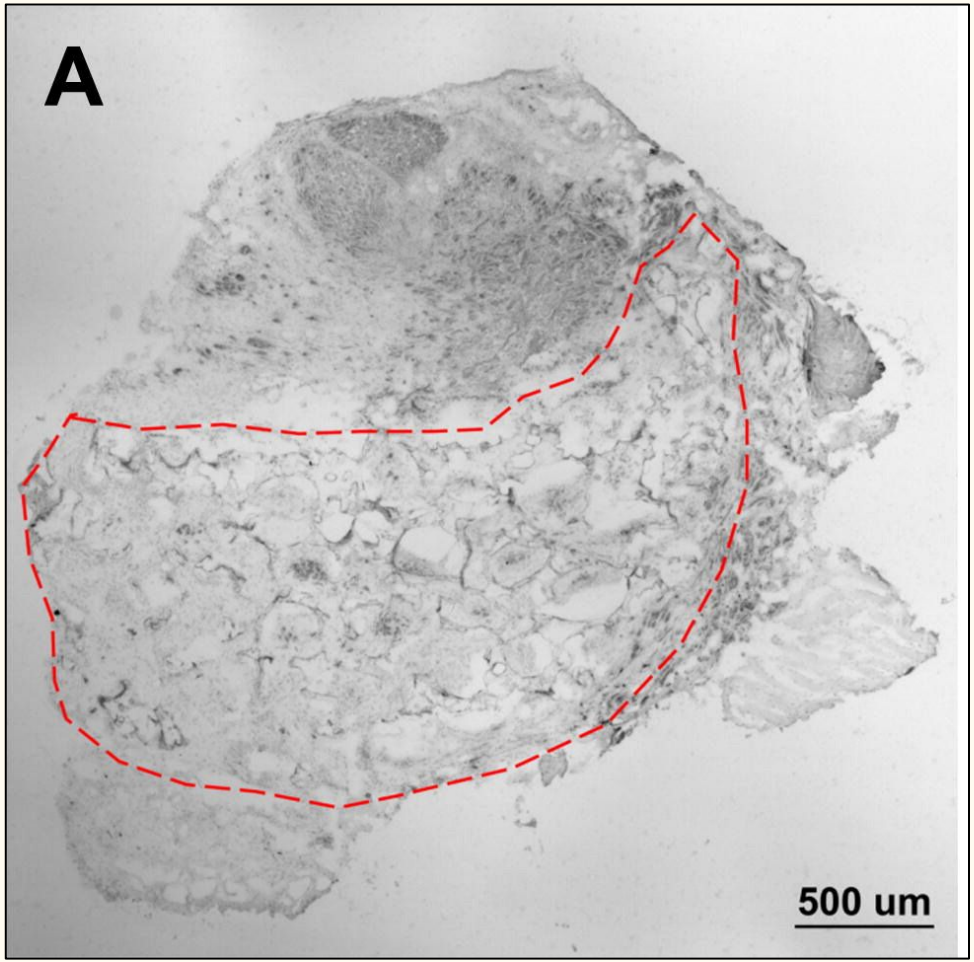
However, type of myelinating cell numbers differed:

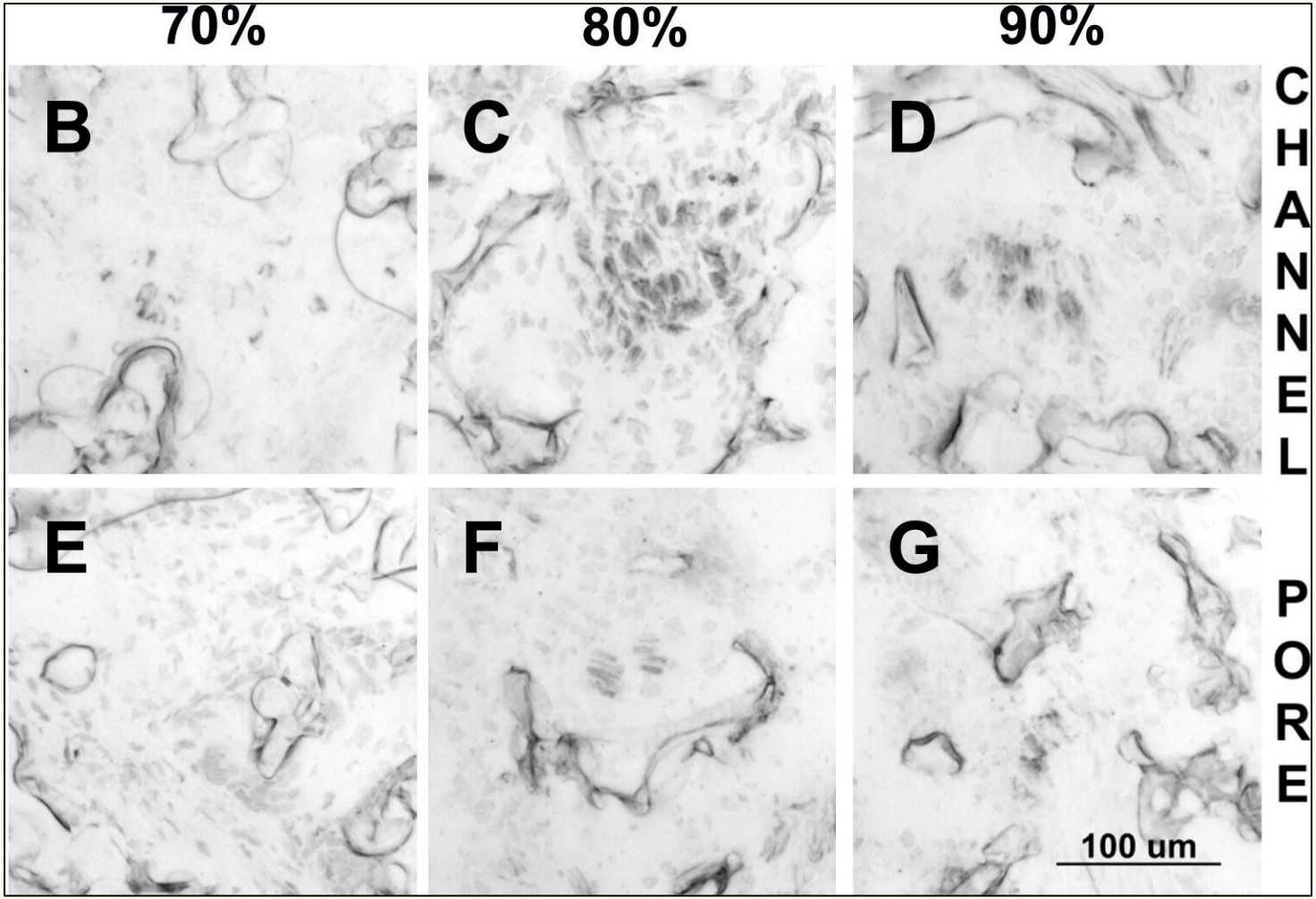
- S-100β+ cells decreased with increasing porosity
- Regenerating neurites and RIP+ cells increased with increasing porosity



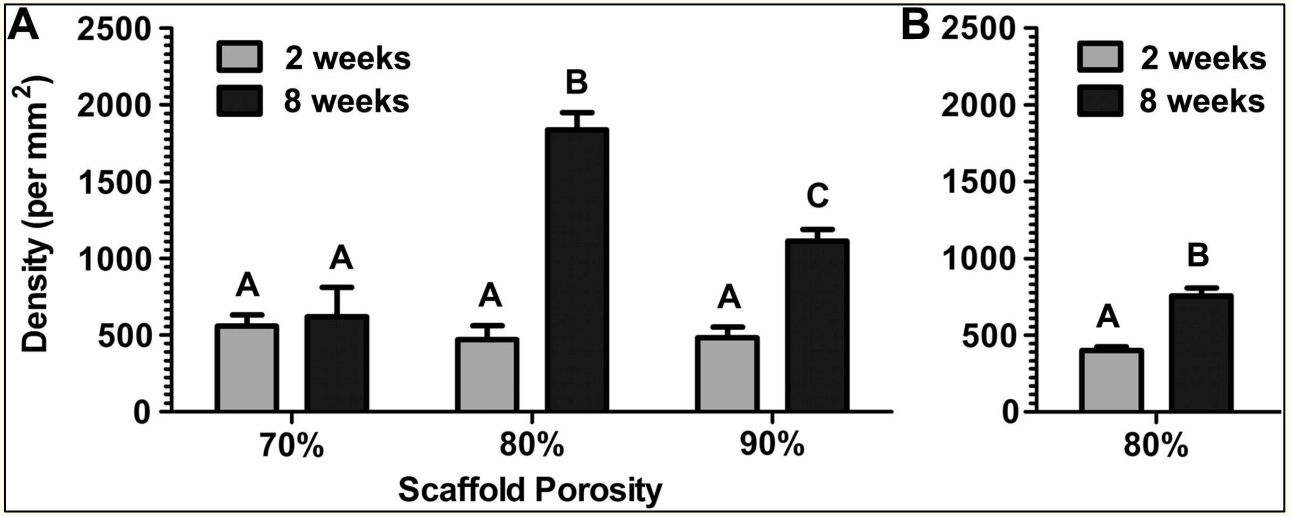
Axon Elongation

- Robust neurite extension in bridge channels of all porosities
- Found at polymer surface




70%
80%
90%
B
C
D
CHANNEL
E
F
G
PORE
100 um

Neurite Density



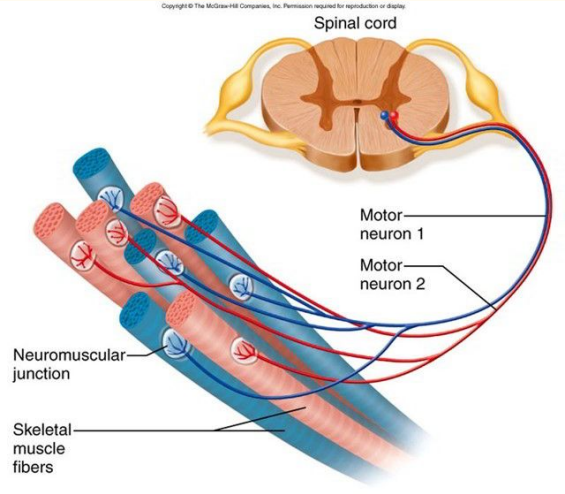
- Similar densities at 2 weeks
- No change in 70% bridge
- 80/90% significantly more dense at 8 weeks
- Figure B: results for mouse implantation
- Similar result as rat at 2 week mark; less dense after 8 weeks

Accumulation of Extracellular Matrix Proteins

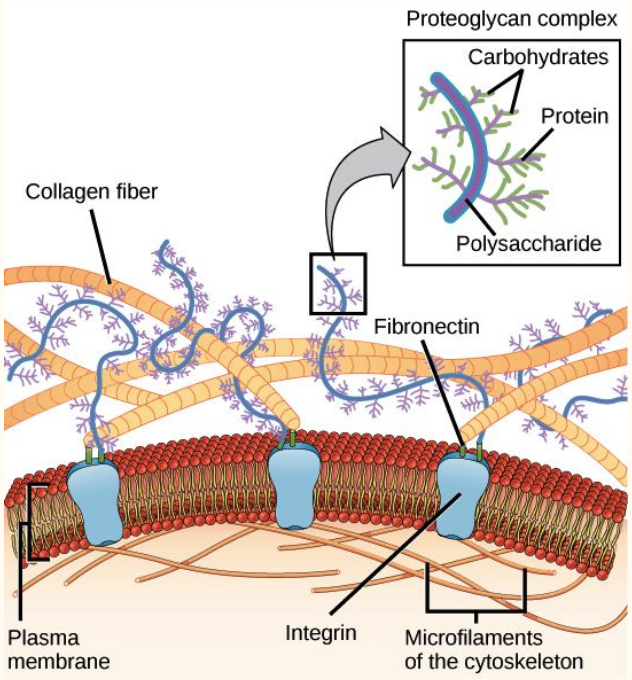
1https://embryology.med.unsw.edu.au/embryology/index.php/Histology_Stains

What are we looking for?

- Evidence of cell residency and neurite growth
- Collagen: fibrous protein in the ECM
  - Staining
    - Collagen will be **blue**
    - Smooth and skeletal muscle cells are **red**[1]



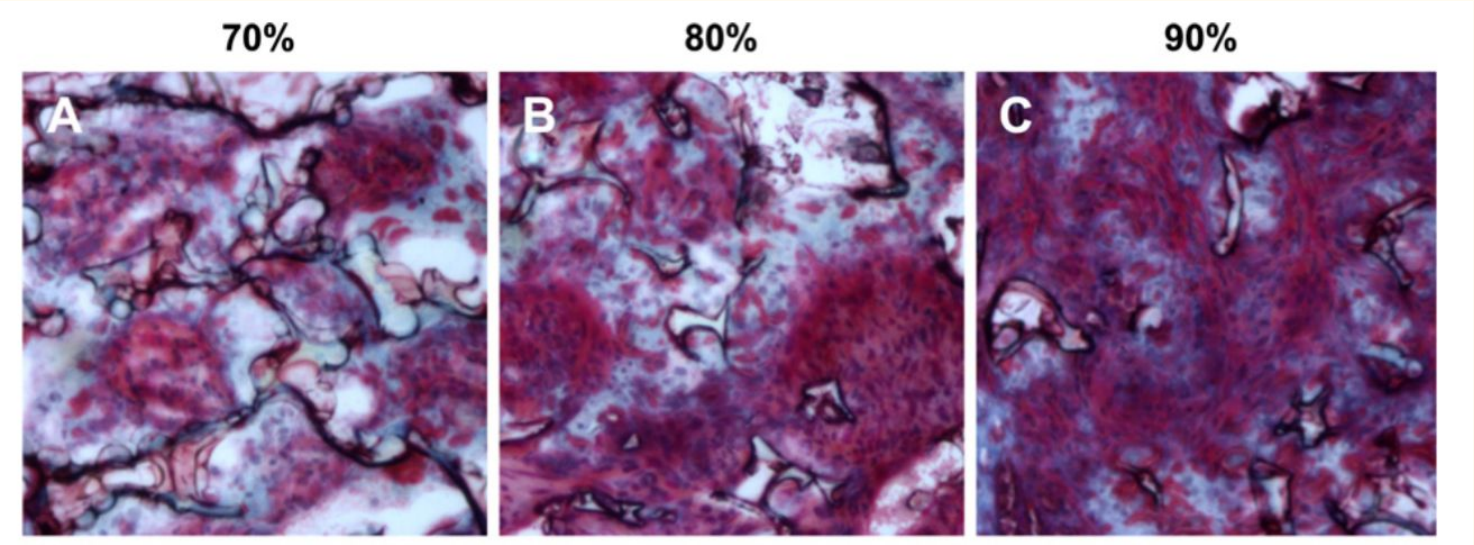
https://www.earthslab.com/physiology/structure-skeletal-muscle/



https://www.khanacademy.org/science/biology/structure-of-a-cell/cytoskeleton-junctions-and-extracellular-structures/a/the-extracellular-matrix-and-cell-wall

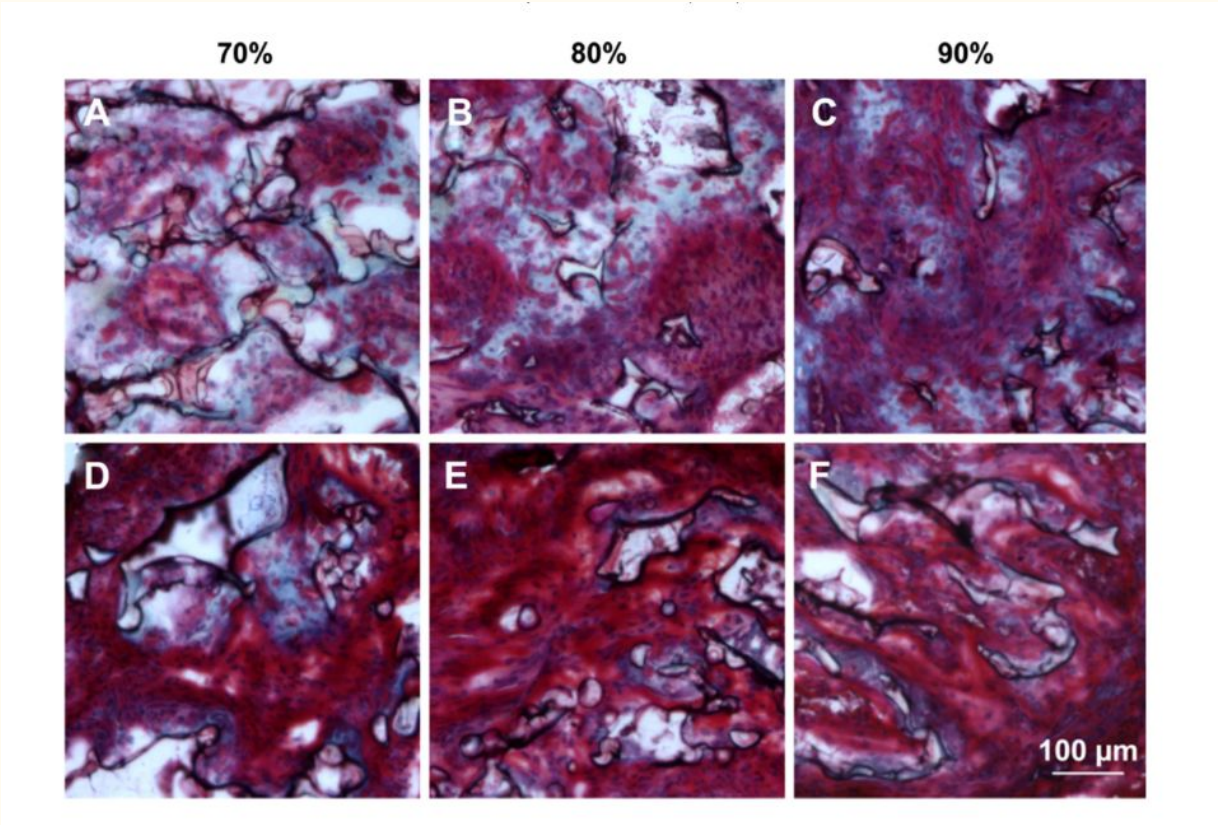
Part 1: 2 weeks after Implantation

- Collagen present in all bridges
- Found in areas near polymer bridge or where polymer was reabsorbed
- ↑ porosity = ↑ collagen area



Part 2: 8 weeks after implantation

- More intense red and blue staining
  - ➢ Suggests more collagen/ECM in those areas
- Collagen area decreases

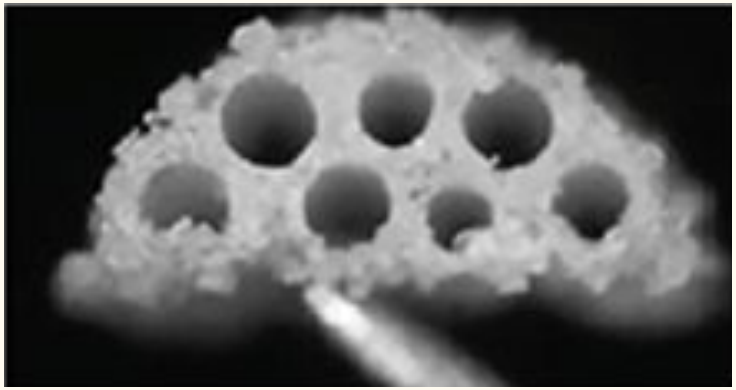


Top row: 2 weeks; bottom row: 8 weeks

Conclusion and Limitations

Gene Delivery Strategies to Promote Spinal Cord Repair - Scientific Figure on ResearchGate.

Conclusion



- **Goal:** determine effect of channels and pores in bridges on neurite development
  - Specifically for rat and mouse spinal cord injury
- Neurite growth present across all porosities
  - 70% porosity did not encourage axon elongation
- Neurite and oligodendrocyte presence ↑ at higher porosities
  - Pore network allowed for other cell infiltration (ie. macrophages)
- Myelination didn't ↑ at higher porosities
- **Overall**, bridge architecture fosters neurite regeneration after spinal cord injuries

Future Research/Limitations

- Things to ask in the future:
  - When does porosity become too much?
  - Implanting stem cells?

- Limitations:
  - Human models vs. rats
  - Long term treatment?

Discussion Questions

1. Review: Neurite and oligodendrocyte presence increased at higher porosity, while myelination did not. Why is this result unusual?
2. What are some other applications of a multiple-channel, porous bridge, inside and outside of healthcare?
3. This study demonstrated that the bridge architecture fosters neurite regeneration but doesn't address the functionality or effectiveness of these extended neurites. Why might that be an issue for future applications of these bridges in human patients?
4. What might be some concerns regarding the stability and longevity of these bridges?