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School of
Biomedical Engineering,
Science and Health Systems

Suture Silk Scaffold to Promote Spinal Cord Repair Through Directional Guidance

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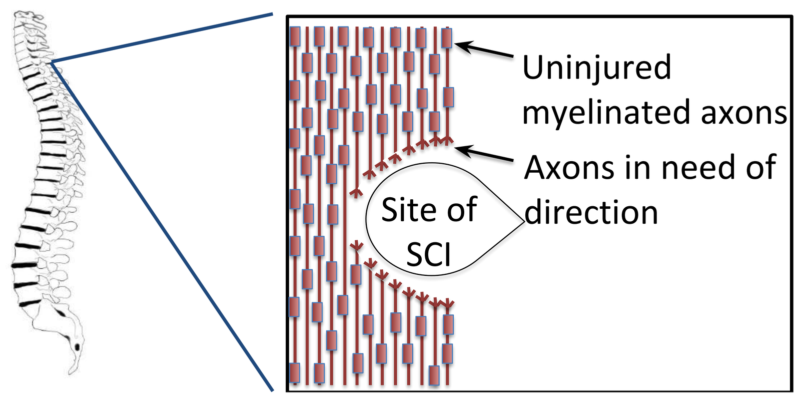
Introduction

- Spinal cord injury (SCI) affects 347,000 people in the USA [1]
- Minimal regenerative and functional recovery due to non-permissive tissue environment [2]



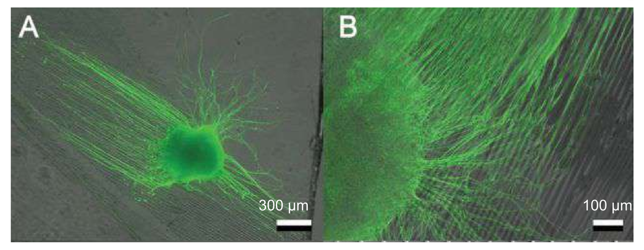
- Research has suggested that axons may still be capable of linear aligned regeneration when given a growth permissive scaffold that provides directional guidance for axons [3] [4]

Existing Scaffold Solutions

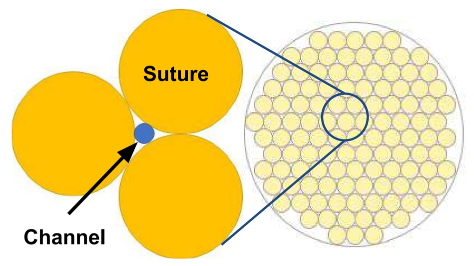
Method	Pros	Cons
Freeze drying	Highly porous structure	Random distribution of pores
Mold casting	Simple, inexpensive	Inability to fabricate an intricate microstructure
Heat compression	Versatile for many polymers	Difficult to control fiber assembly

Design Rationale

- Neurites will adhere to and grow along silk fibroin [6]
- Neurites will grow in the direction guidance is provided [4]



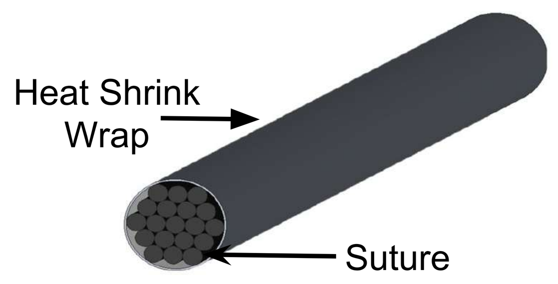
- Packing model shows presence of channel sizes

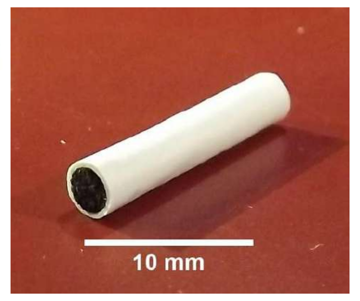


Gauge	Diameter (µm)	Channel Size (µm)	Neurites Accommodated
2	500	77.3	~10
4-0	150	23.6	~3

Proposed Solutions

A 3 mm diameter scaffold constructed from gauge 2 braided silk suture with channels constrained by PET heat shrink tubing to promote axon regeneration

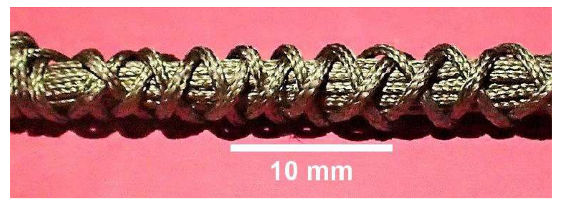




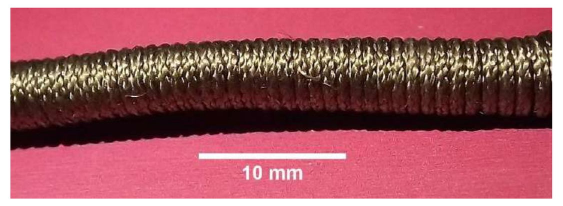
Heat Shrink Tubing

Alternative Solutions

Braid



Spiral Bound

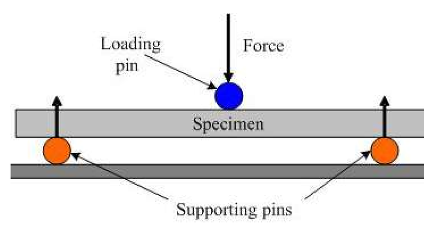


Gauge 4-0 suture will also be tested as lower bound for all scaffold designs

Design Criteria

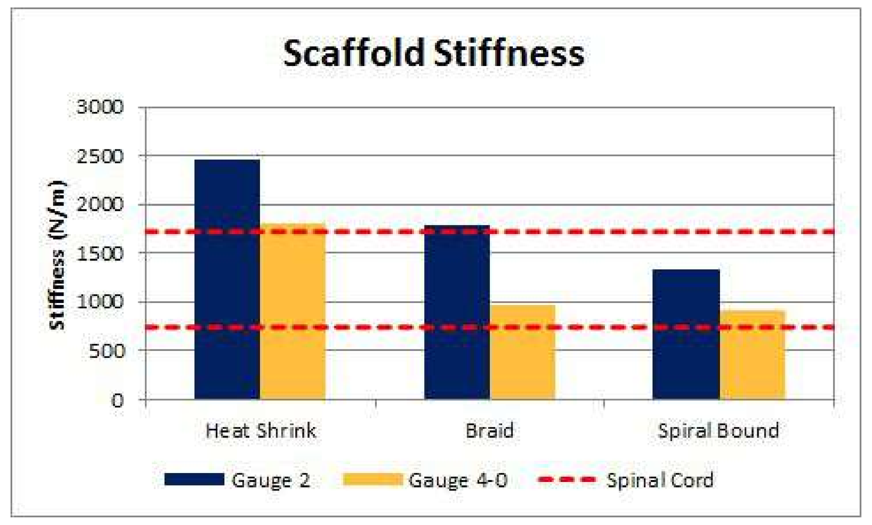
- Mechanical Testing
  - Stiffness (1230 ± 490 N/m), Flexural Modulus (0.74 ± 0.14 MPa) [10][11]
    - Three point bend test
    - Scaffold properties compared to native tissue to prevent damage
- Channel Size (23-77 µm)
  - Cross sections analyzed with SEM and ImageJ
  - Minimum and maximum passage to allow sufficient size for neurite growth
- Suture Swelling (<5% of suture diameter) [9]
  - Suture submerged in PBS for 48 hours
  - Swelling of suture must not cause occlusion of the channels
- Directional Neurite Growth (100 µm growth and no more than ±15° deviation from channel neutral axis) [7] [8]
  - Analyze DRG growth on aligned suture post 48hr incubation
  - Must exceed DRG neurite growth without channel guidance

Mechanical Test Results



Three point bend apparatus

Scaffold Design	Stiffness (N/m)		Flexural Modulus (MPa)	
	Gauge 2	Gauge 4-0	Gauge 2	Gauge 4-0
Heat Shrink	2450	1797	58.86	254.56
Braid	1790	972.3	53.95	150.23
Spiral Bound	1340	909.5	57.20	403.89
Design Criteria	1230 ± 490		0.74 ± 0.14	



Stiffness requirements of 1230 ± 490 N/m were met by gauge 2 and 4-0 spiral bound and gauge 4-0 braid scaffolds. No scaffold met the requirement of 0.74 ± 0.14 MPa for flexural modulus.

Channel Size Test Results

Scaffold Design	Measured Channel Size (µm)		
	Gauge 2	Gauge 4-0	Monofilament Gauge 0
Heat Shrink	40.74	49.28	66.74
Braid	110.78	24.64	N/A
Spiral Bound	81.75	36.99	N/A
Design Criteria	76.65	23.25	54.25

Results show that the measured channel size for gauge 2 spiral bound and gauge 4-0 braided were similar to what was expected based on the packing model

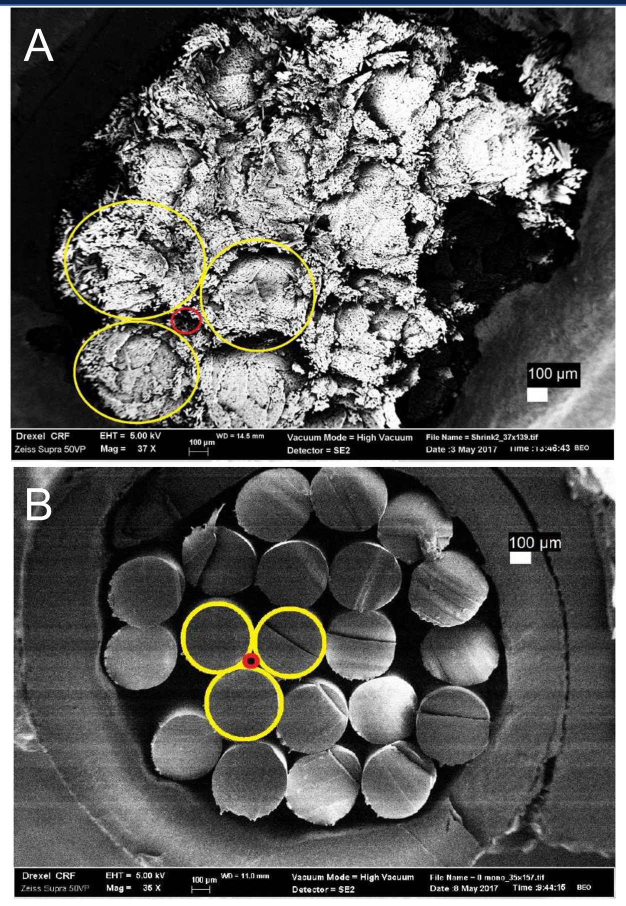


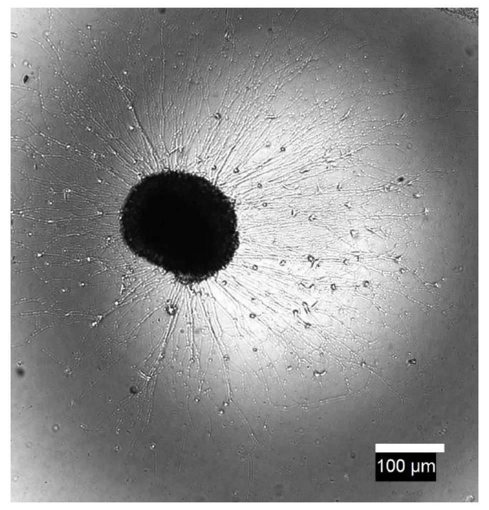
Figure A: SEM image showing channel size (red circle) of shrink wrap tubing with gauge 2 silk suture (yellow circle) **Figure B:** SEM image showing channel size (red circle) of shrink wrap tubing with gauge 0 nylon monofilament (yellow circle)

Suture Swelling Test Results

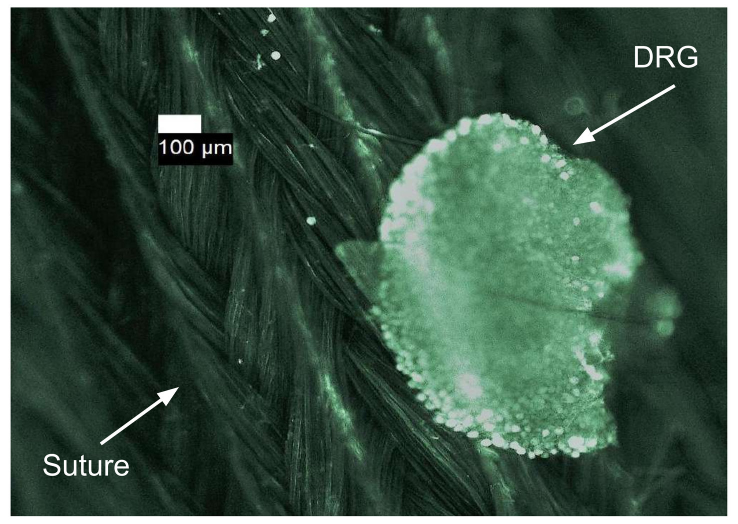
Suture Gauge	Suture Diameter		Paired t-test P Value (one tail)
	Pre-Swelling	48hr Swelling	
2	672 µm	708 µm	0.635
4-0	245 µm	253 µm	0.171

Gauge 2 suture did not meet the requirement of <5% (705.6 µm) of suture diameter, while gauge 4-0 did meet the requirement of <5% (257.3 µm) of suture diameter

Neurite Growth Test Results



Growth of unguided DRG neurites on laminin coated glass coverslip



Fluorescent image of DRG on 3-0 PLO/laminin coated suture sample post 48 hour incubation

Results show that DRGs firmly adhered to suture silk, but growth was not observed. Directional neurite growth requirements of 100 µm growth and no more than ±15° deviation from channel neutral axis were not met.

Conclusions and Future Work

- Gauge 4-0 braid design showed most promising test results
  - Met stiffness, channel size and swelling requirements
- Directional growth was not observed on suture silk
  - Processing on suture silk may impede neurite growth
- Cross sectioning of sutures affected channel size measurement
- Strong DRG attachment to silk suture was observed
- Future work
  - Test neurite growth on pure silk fibroin
  - Modify scaffold design to meet mechanical properties

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