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Materials and Schwann cell approaches for repair to nerve injury

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Biomaterials in Medicine: New concepts of drug-free antibacterial therapies
Westminster University

20th July 2016





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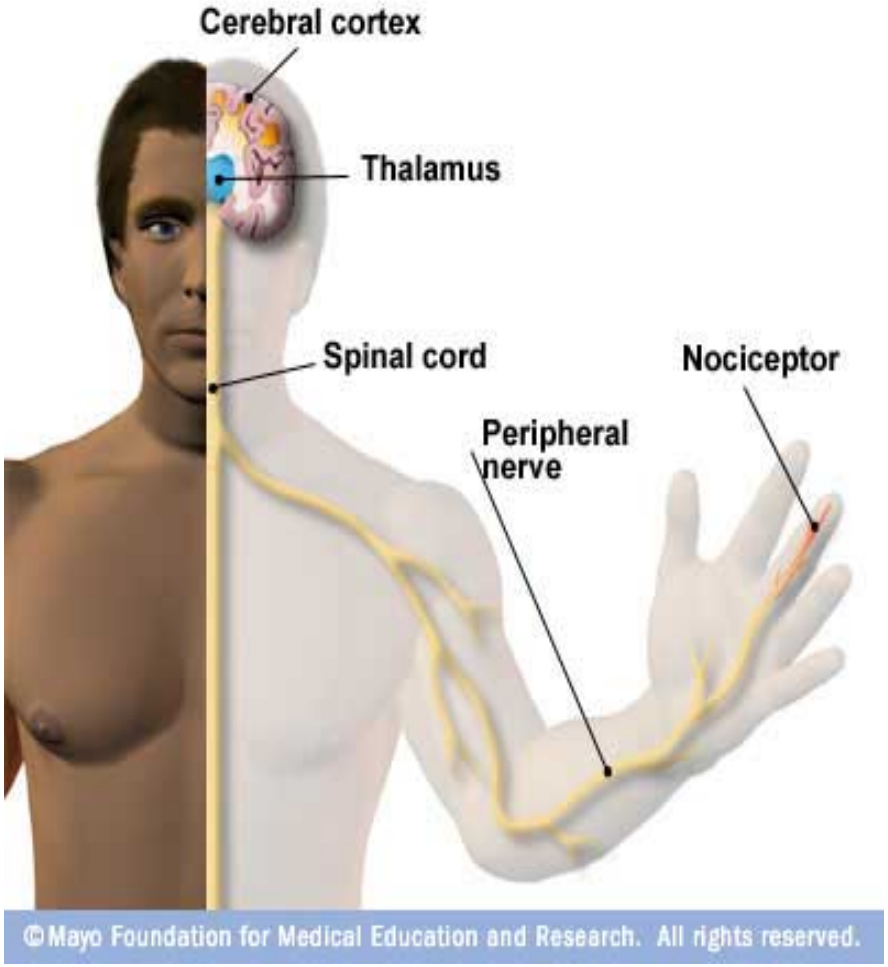
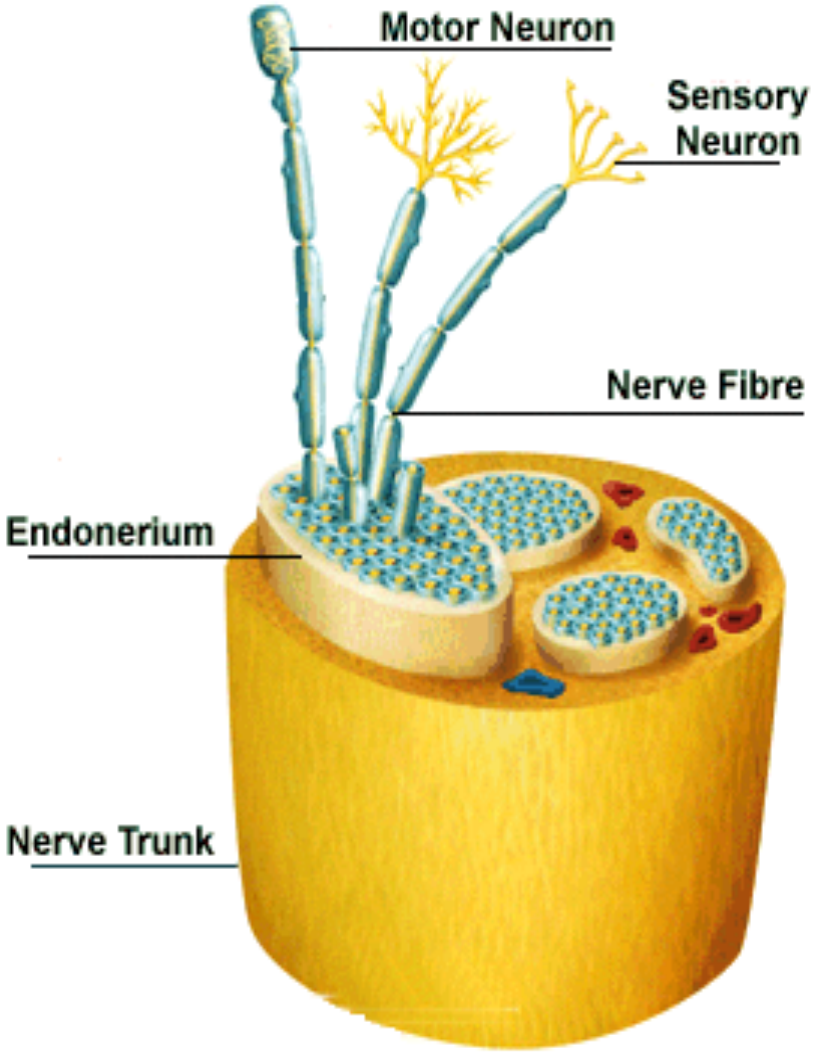
Peripheral nerve





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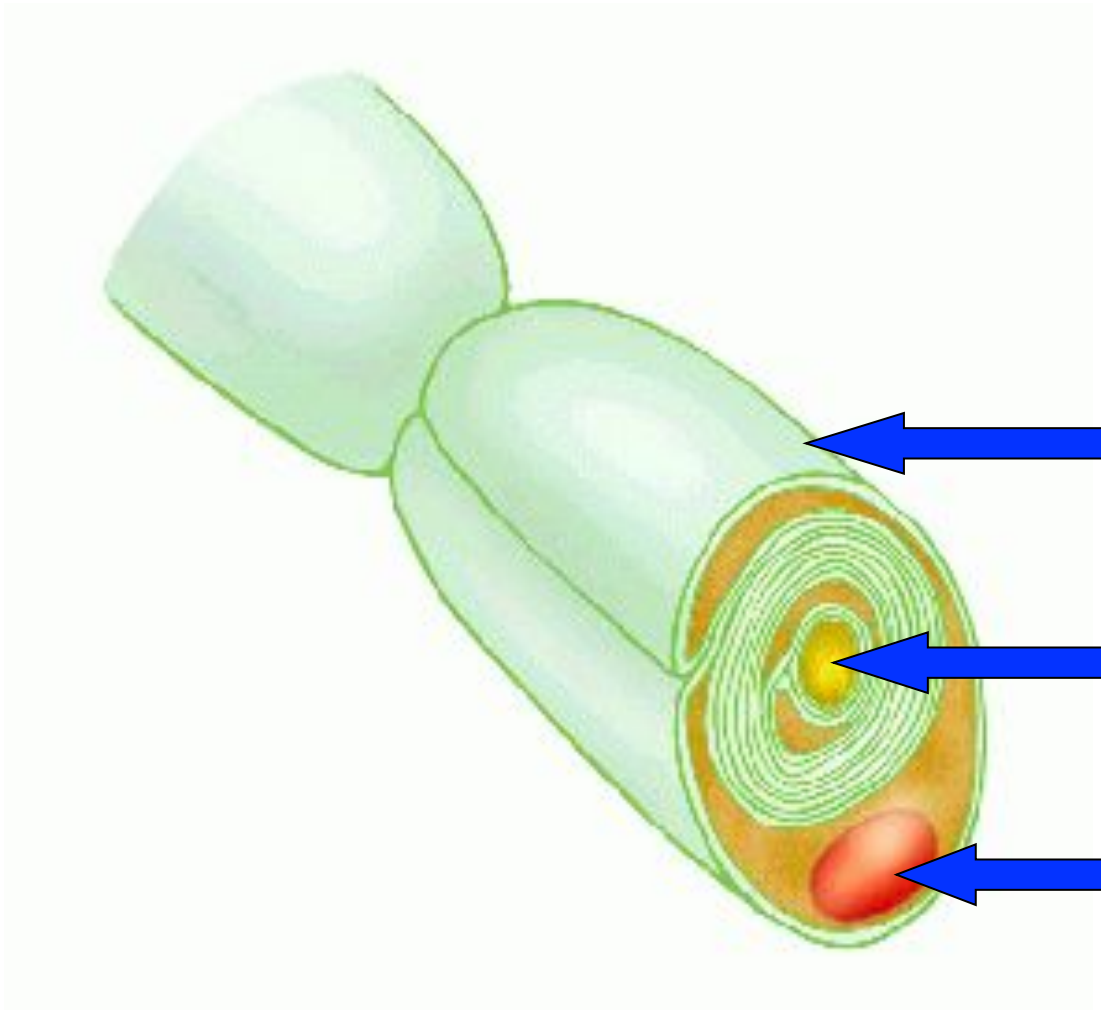
Peripheral nerve axons



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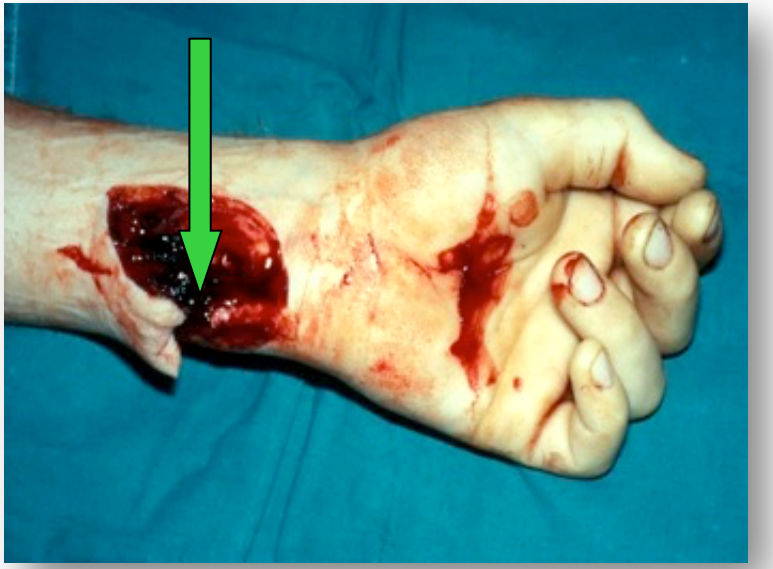
Peripheral nerve injury

- Injuries to the peripheral nervous system
  - USA 360 000 injuries per year
  - Europe 300 000 injuries per year
- Typically result from acute trauma
  - Road traffic accidents
  - Work accidents
  - Domestic accidents



- 80-90% patients show permanent sensory or motor deficit following nerve repair
- Have profound social and economic costs

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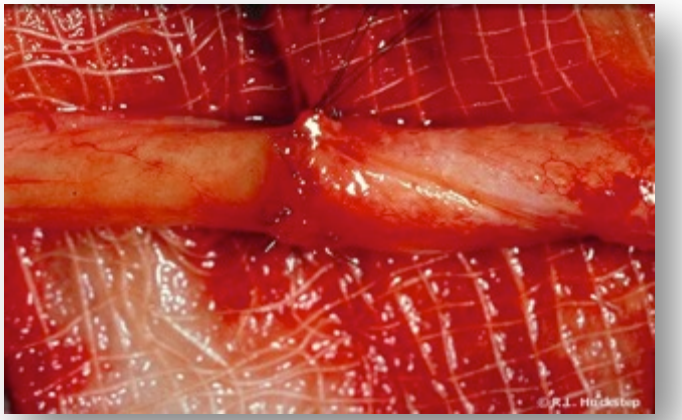
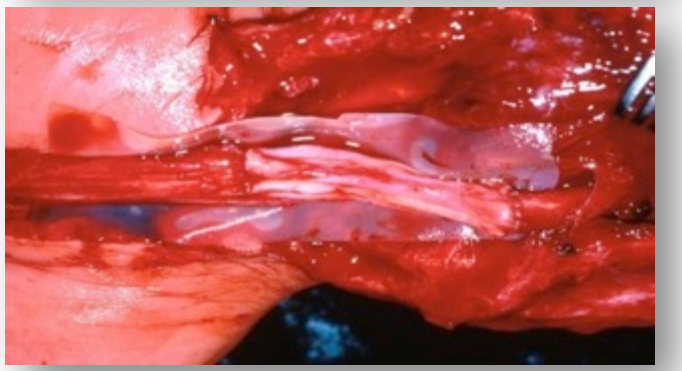
Three main clinical strategies to repair gap injuries

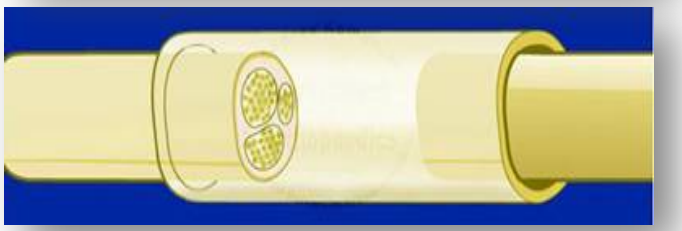
1. **Suturing together proximal and distal ends**
   - + ‘Clean' transection injury
   - - Tension in sutures

2. **Autografting**
   - + Good reinnervation
   - - Donor site morbidity

3. **Nerve guidance conduits**
   - + Biocompatible materials
   - - Primitive design
   - - Limited regeneration







www.bgsm.edu/ortho/brachial_plexus_menu.htm

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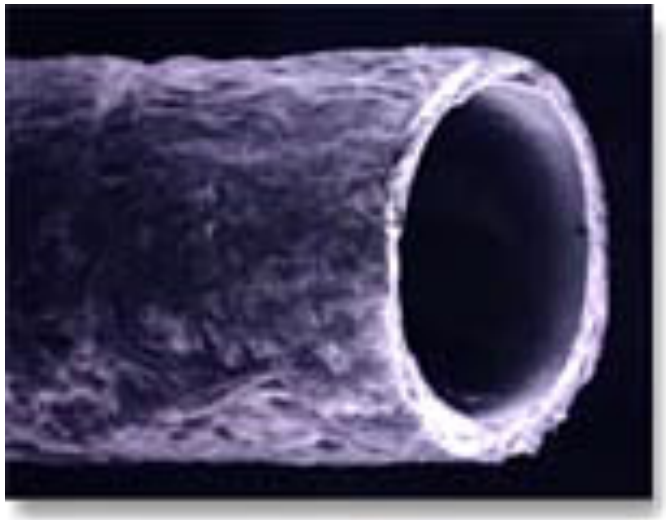
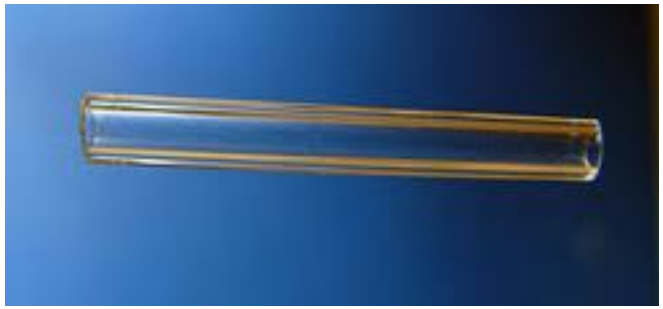
Nerve Guidance Channels



- Collagen - Integra Life Sciences NeuraGen™ nerve guide
- Silicone - SaluMedica's SaluBridge™ nerve cuff



- PGA – Neurotube (Synovis)
- PLLA/PCL – Neurolac (Polyganics)

Bell JHA and Haycock JW (2012). Next generation nerve guides - materials, fabrication, growth factors and cell delivery. ***Tissue Engineering*** 18(2):116-28

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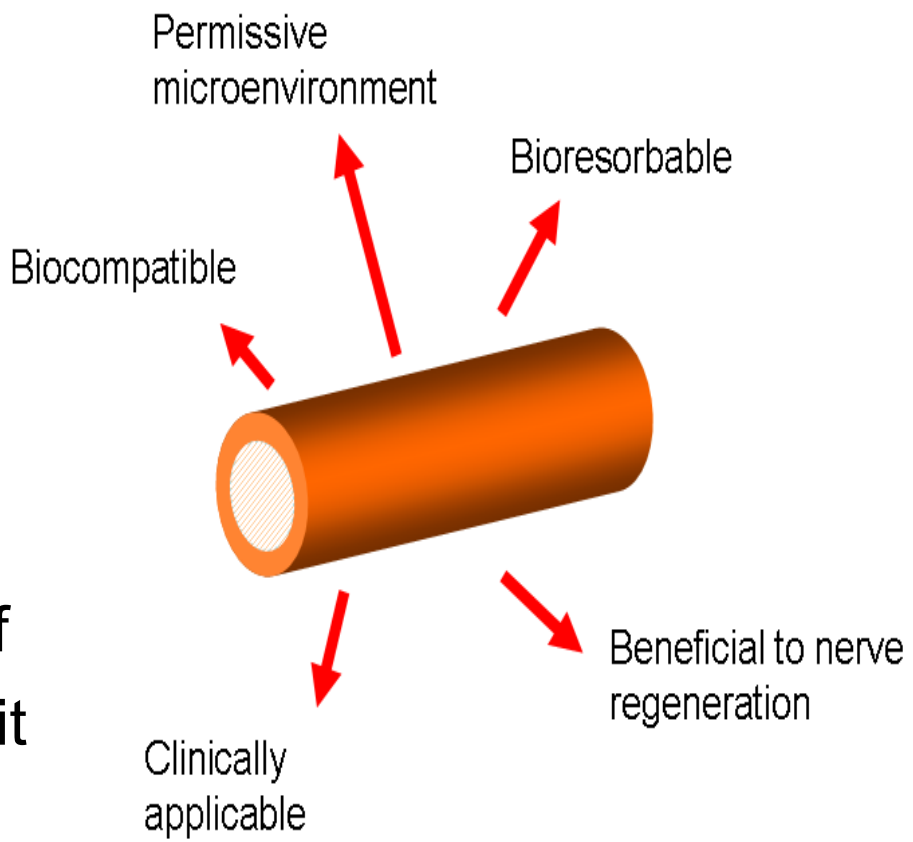
Present strategies for repairing peripheral nerve

- To increase regeneration distance
- To improve extent and effectiveness of reinnervation
- Involves a combination of
  1) Nerve guidance conduit
  2) Schwann cells



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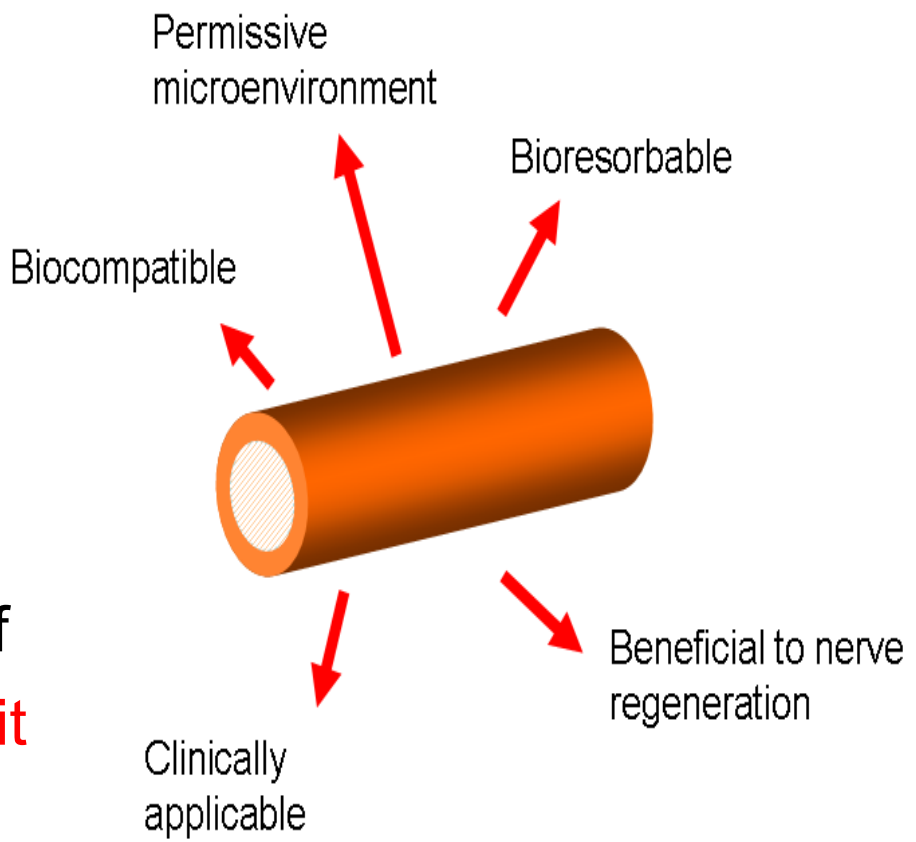

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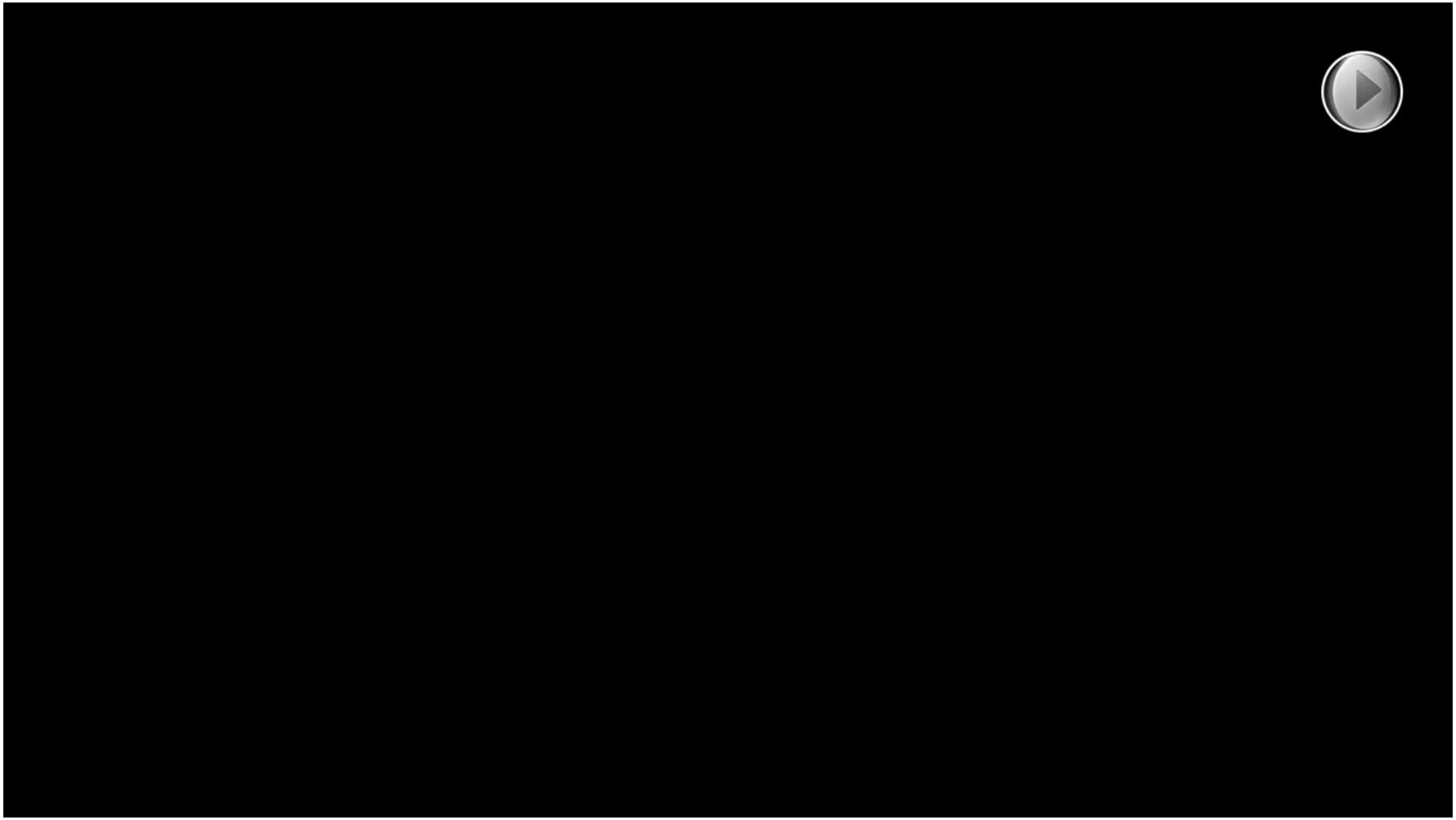

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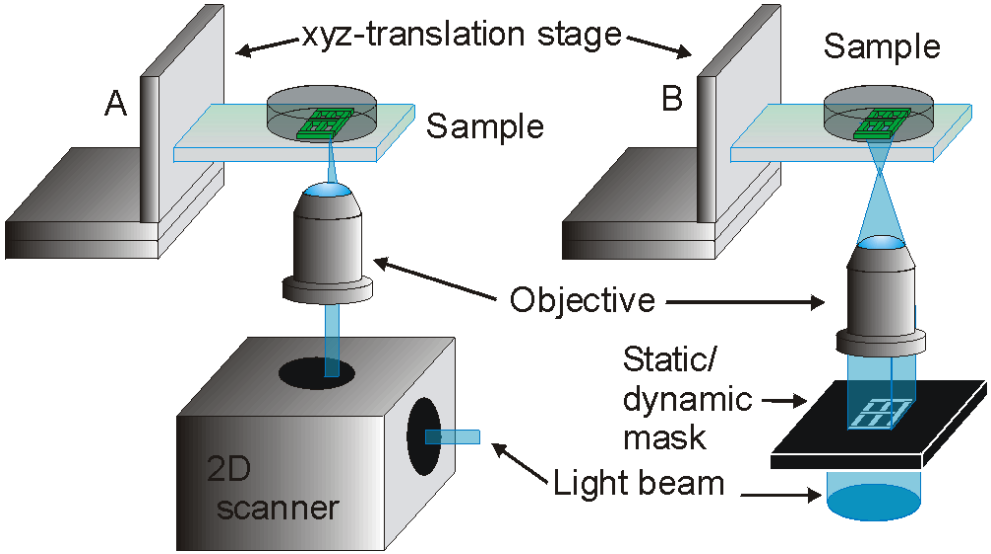
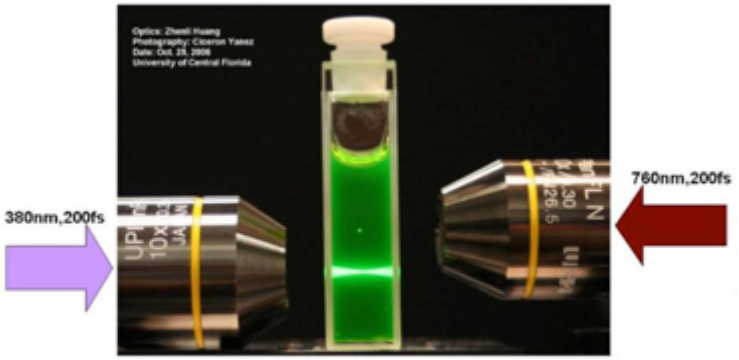
Making a scaffold precisely

Micro-stereolithography





- Construction of 3D objects from photo-curable materials
- UV laser: 3D objects with 10 - 50 µm resolution
- 2-photon polymerisation: structures with <10 µm resolution

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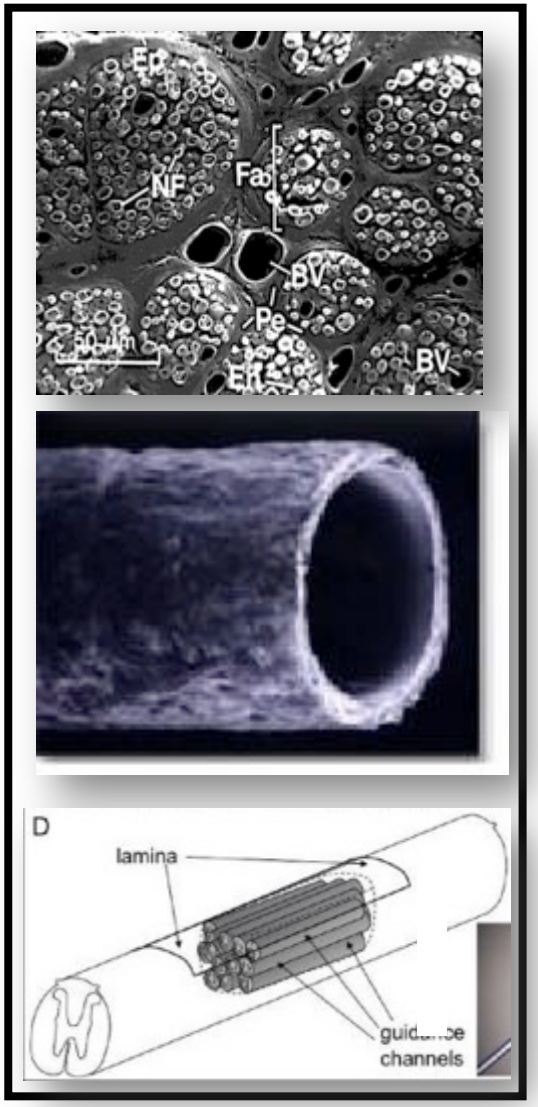
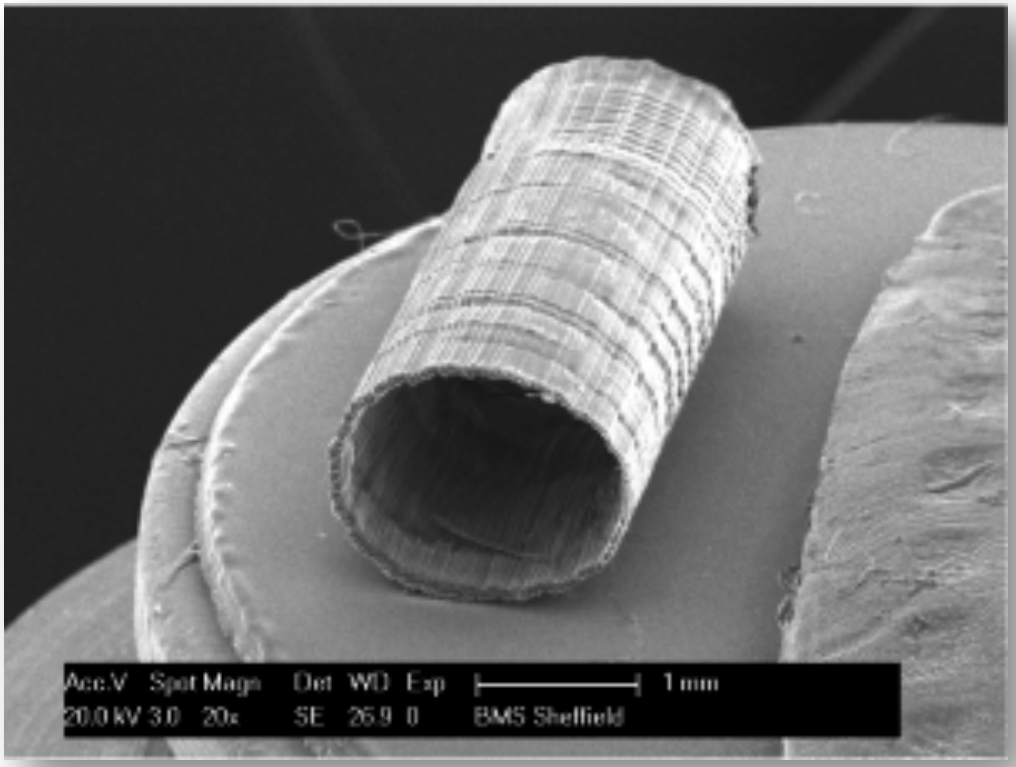
Making a scaffold precisely

Micro-stereolithography



1. Manufacture of NGCs with PEG, PLA or PCL

2. Incorporate internal structure within the tube to improve regeneration



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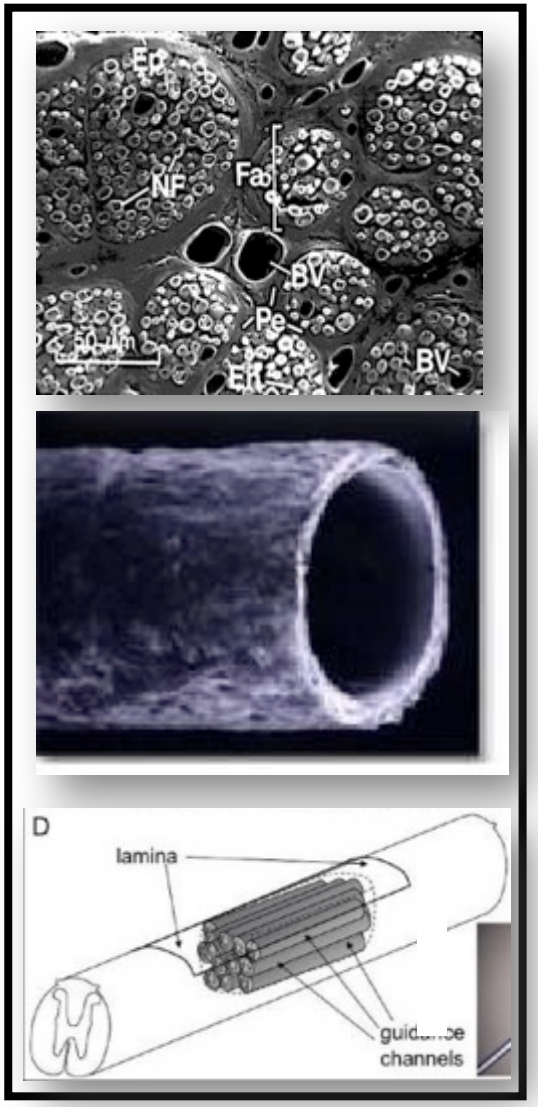

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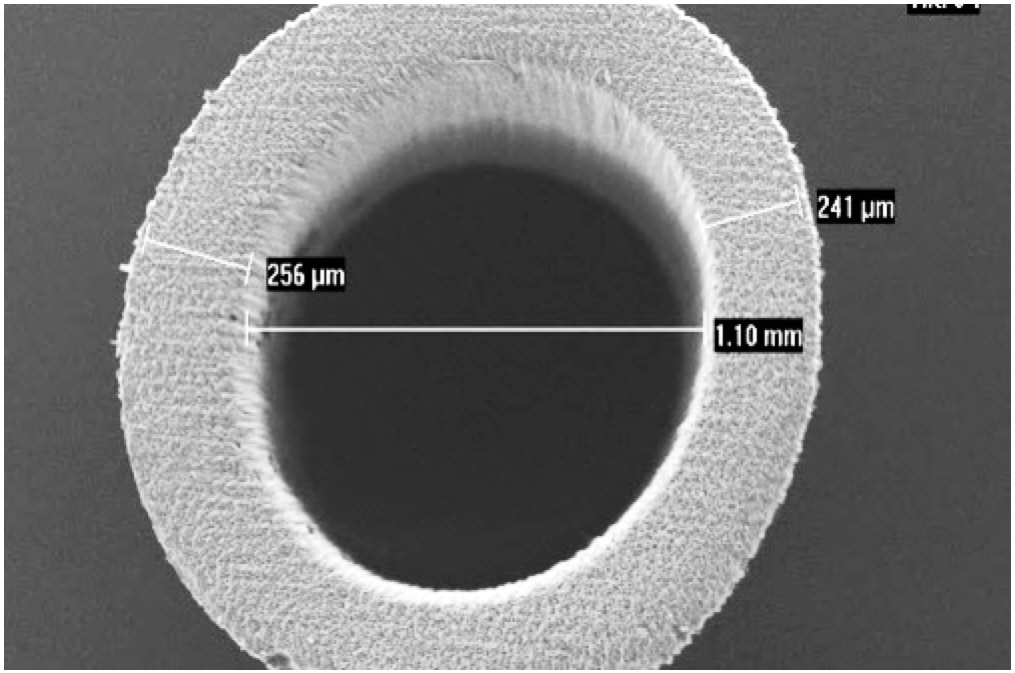

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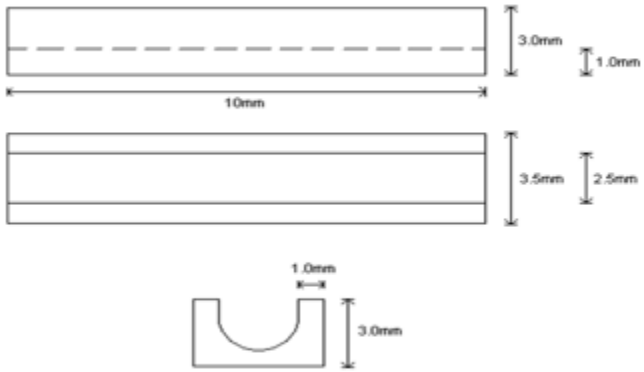
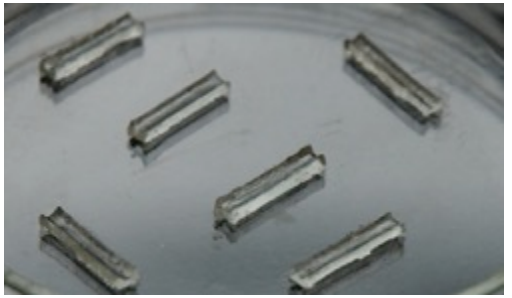
Channels for in vitro culture

- Channels allow the placement of a rat derived explant DRG into the center, in vitro culture and subsequent immunolabelling and imaging
- Produced by irradiating photocurable resin to channel cross section
- Mounted into 6-well plate for in vitro culture
- Essential to ensure smooth uniform structure





Channels before mounting

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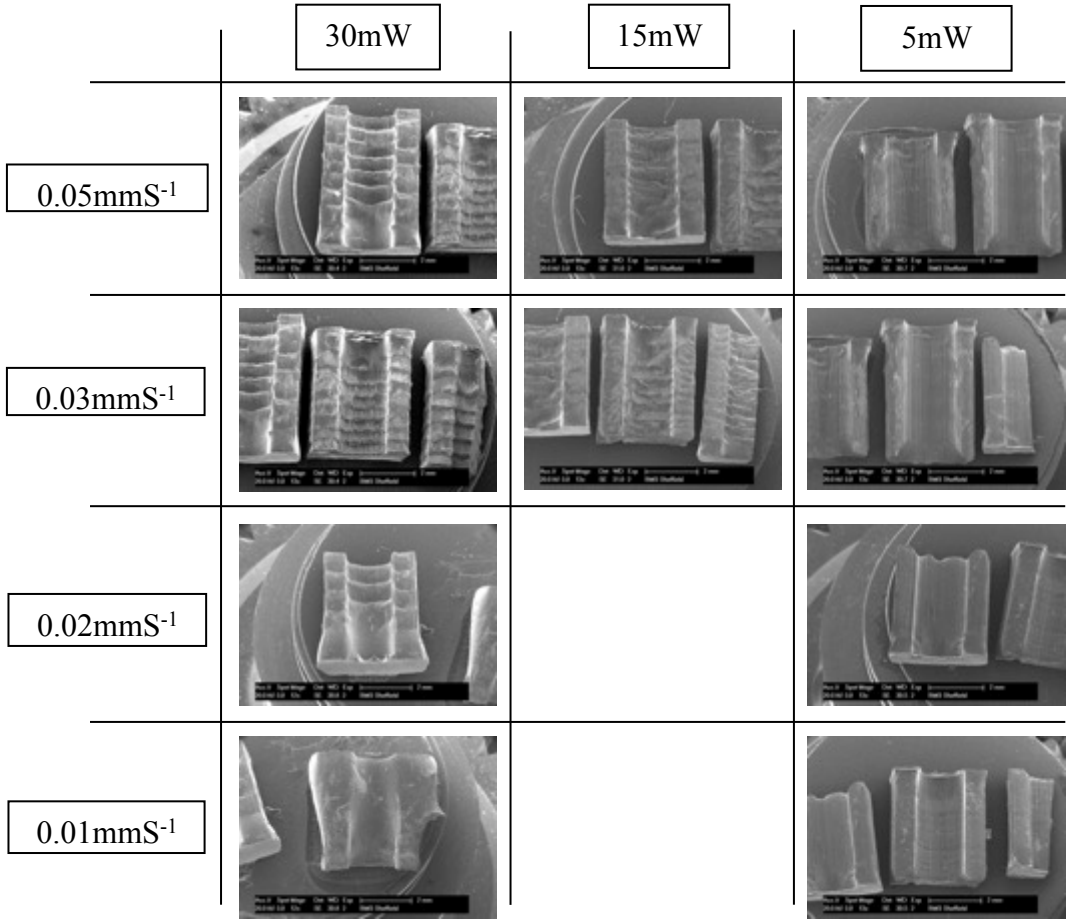
DRG channel structures

- SEM micrographs of poly(ethylene glycol) channels produced by 405nm micro-stereolithography
- Varying laser power of 30mW, 15mW and 5mW
- Varying z-axis translation velocities of:
  - 0.05 mm/s
  - 0.03 mm/s
  - 0.02 mm/s
  - 0.01 mm/s



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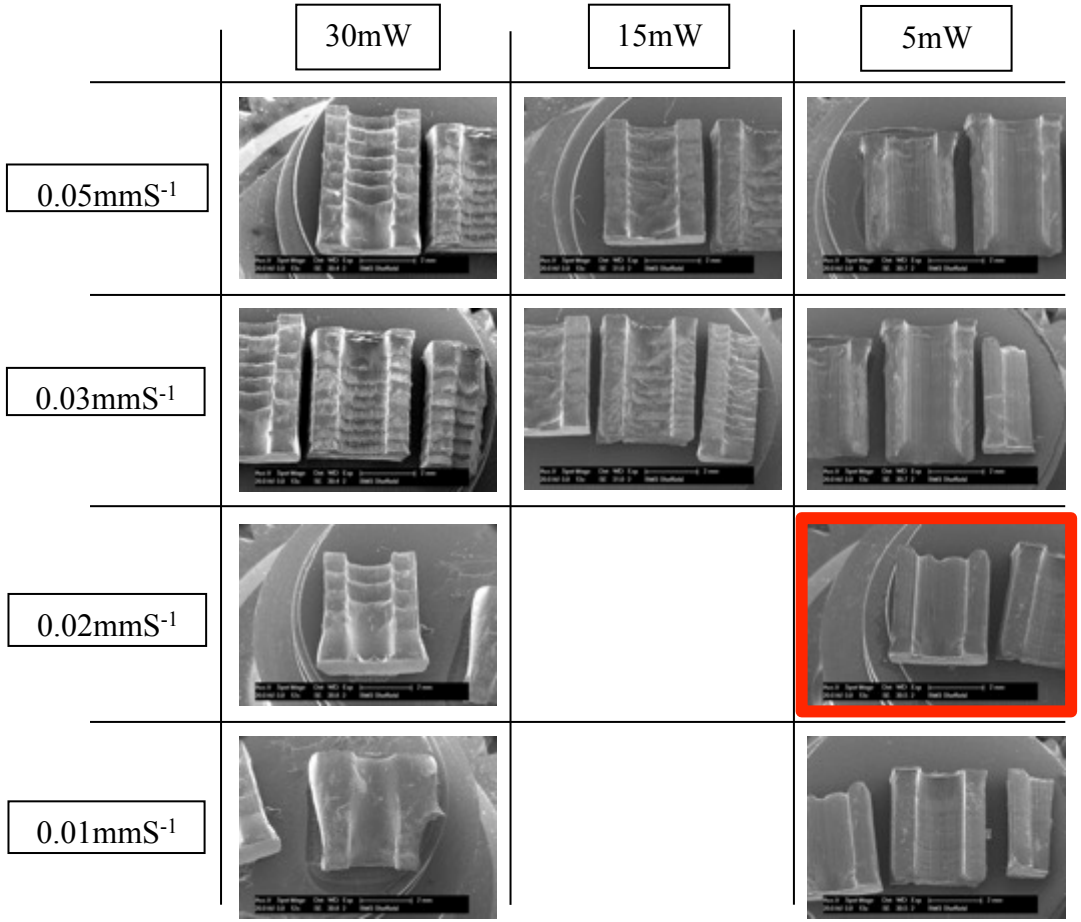

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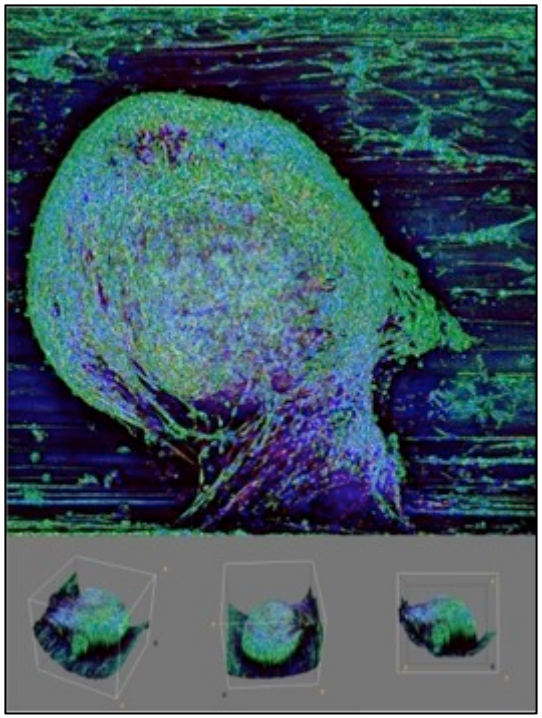
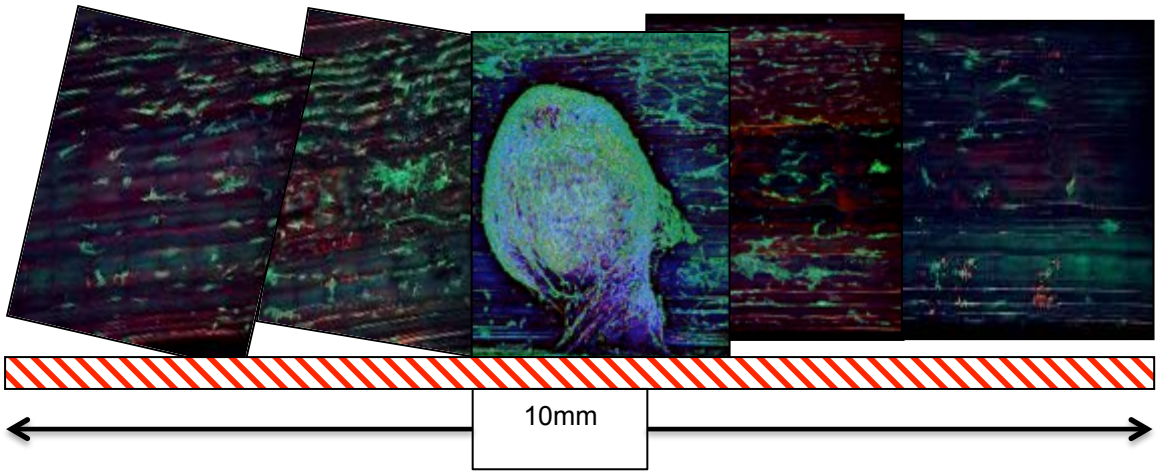
Use of in vitro DRG culture for analysis

Dorsal root ganglion were placed within channels and cultured for 14 days.
Degree of cellular outgrowth assessed by immunolabelling and 2-photon imaging.





Green	=	Schwann cells
Red	=	Neuronal cells
Blue	=	Nuclei = all cells

Z-stack converted into 3D using EDF ImageJ plugin

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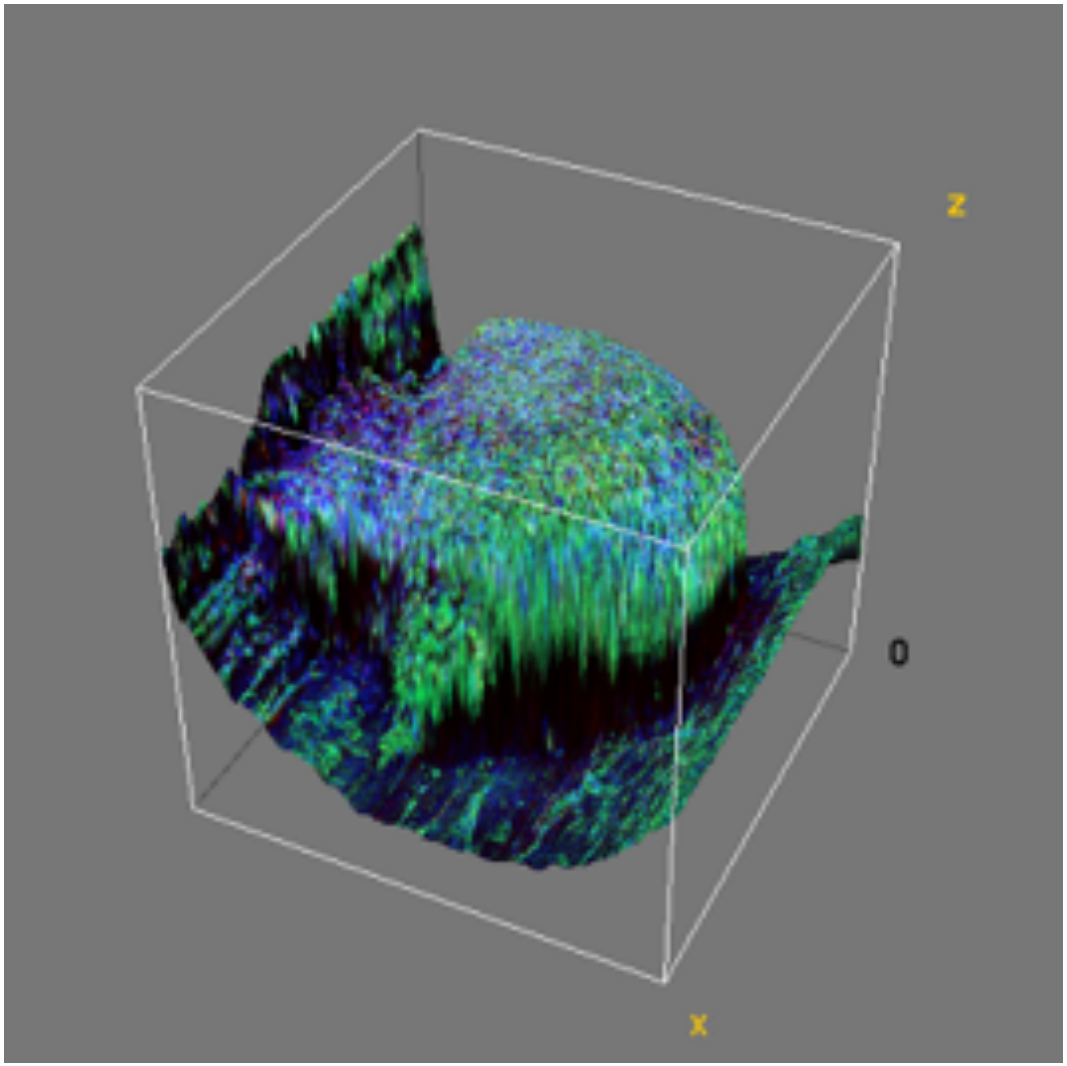
Channels for in vitro DRG culture

2-photon microscopy



Neurites – βIII tubulin

Schwann cells – S100β

Nuclei – DAPI

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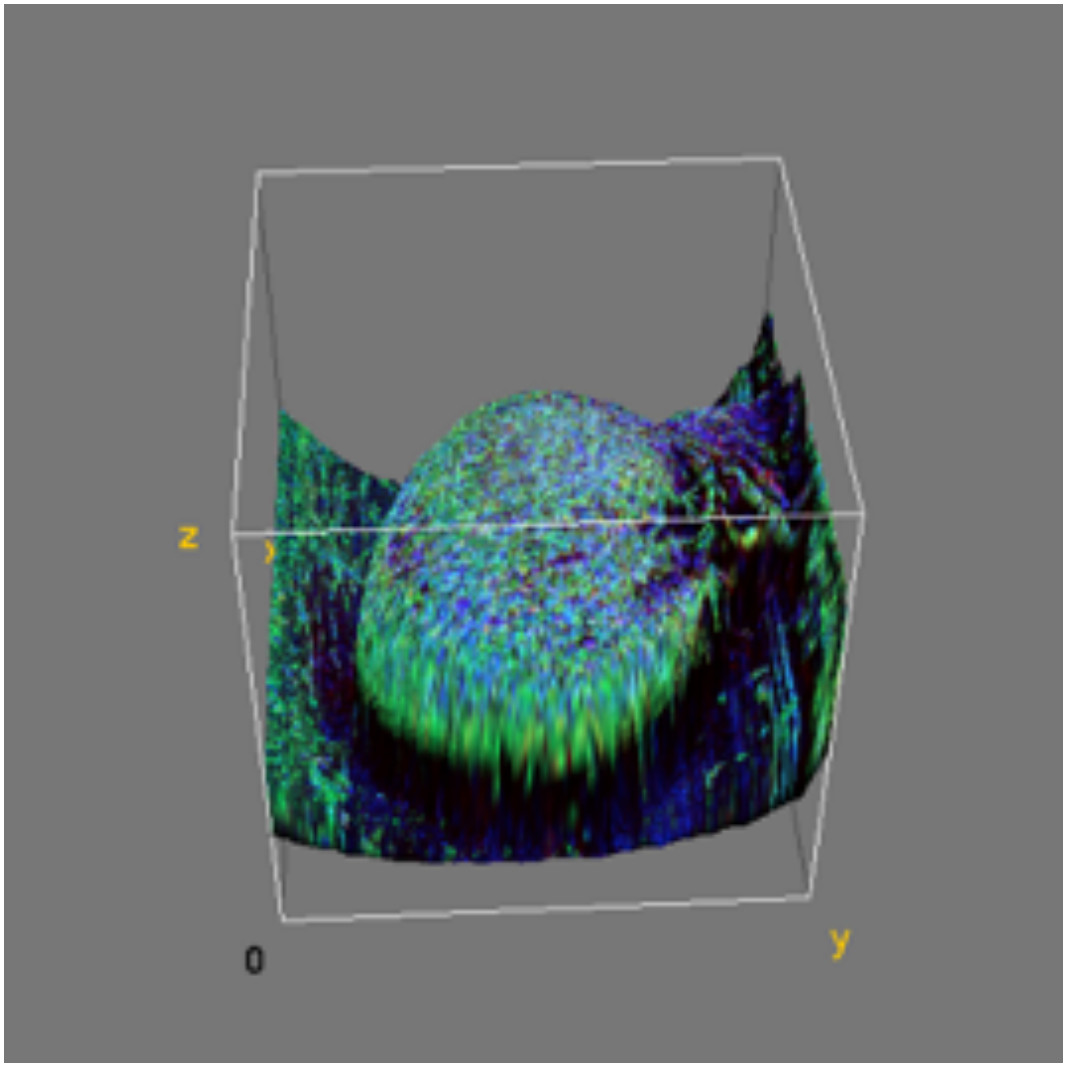

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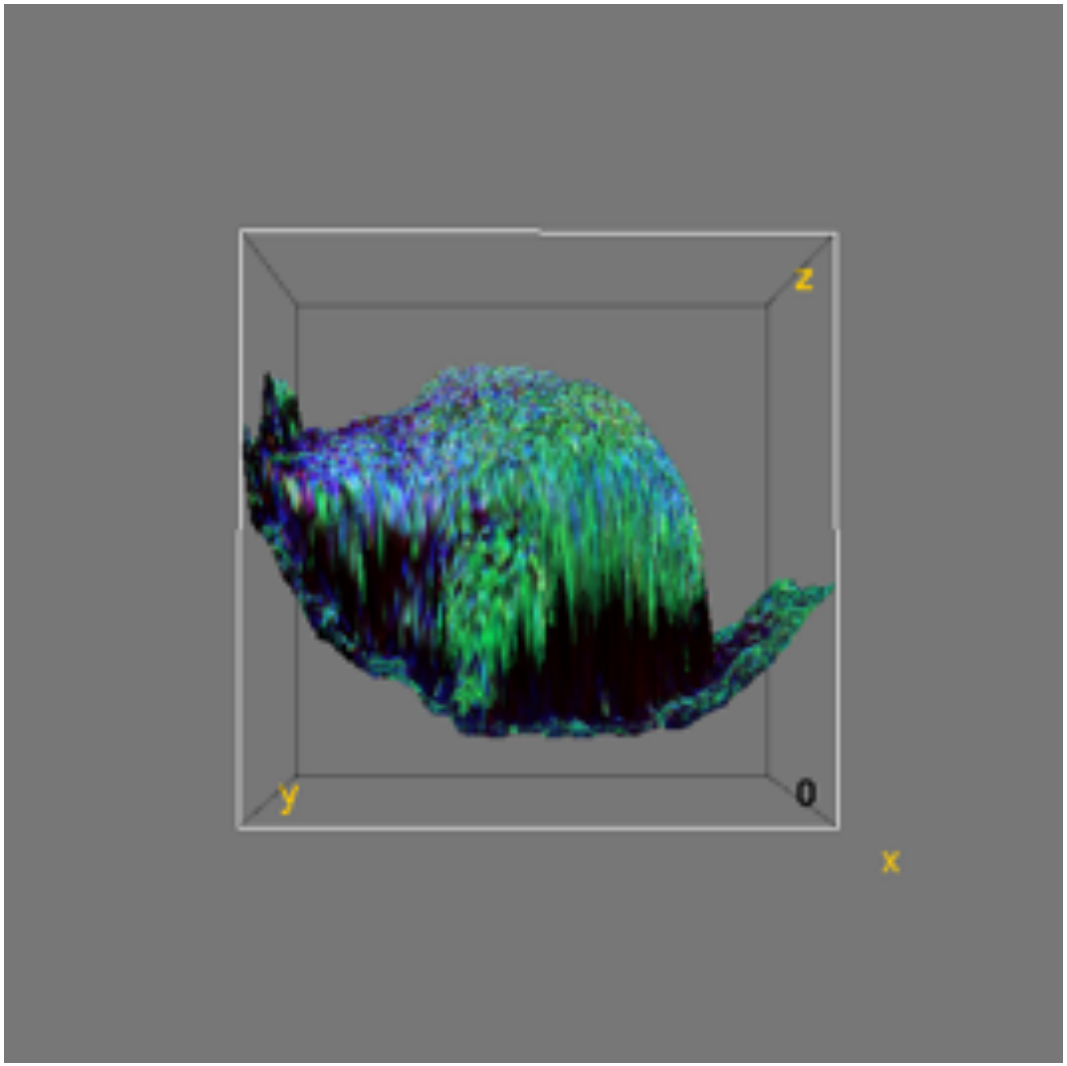

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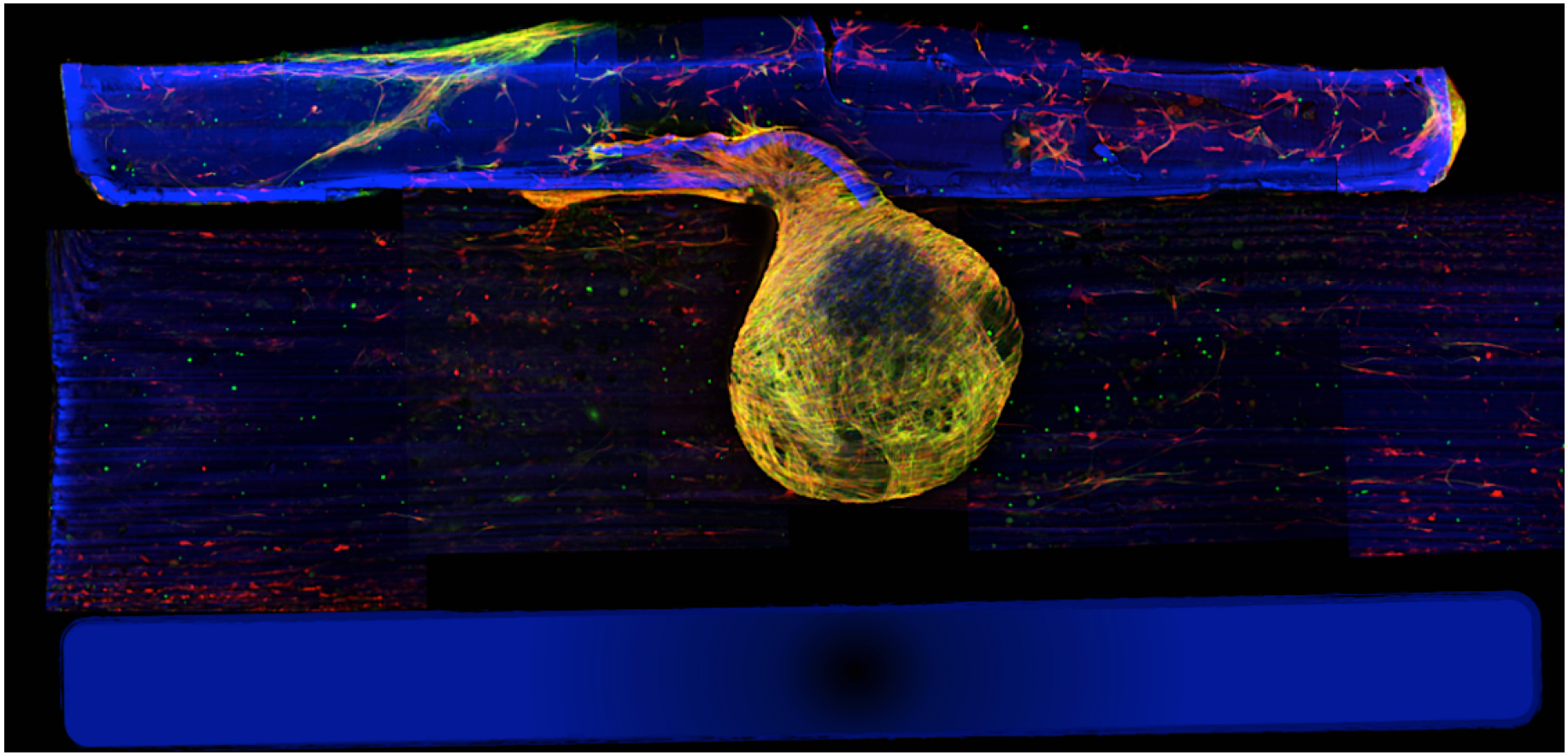

Mr Dharaminder Singh, PhD student (EPSRC), Materials Science & Engineering, University of Sheffield

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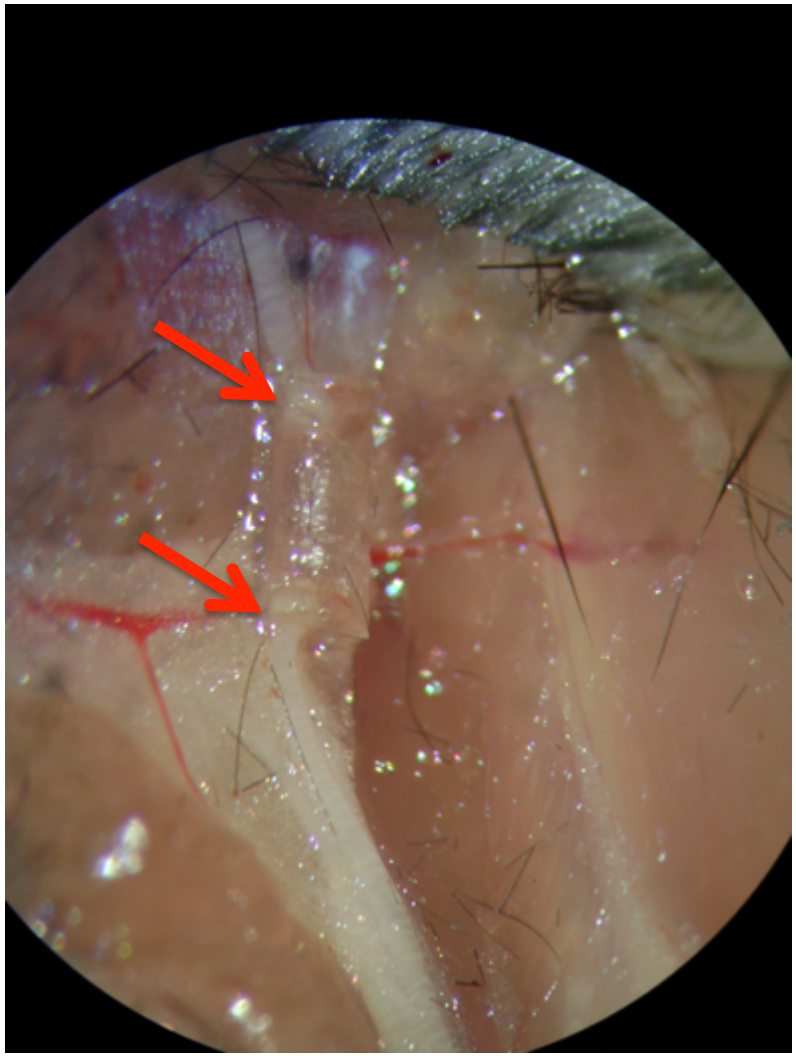
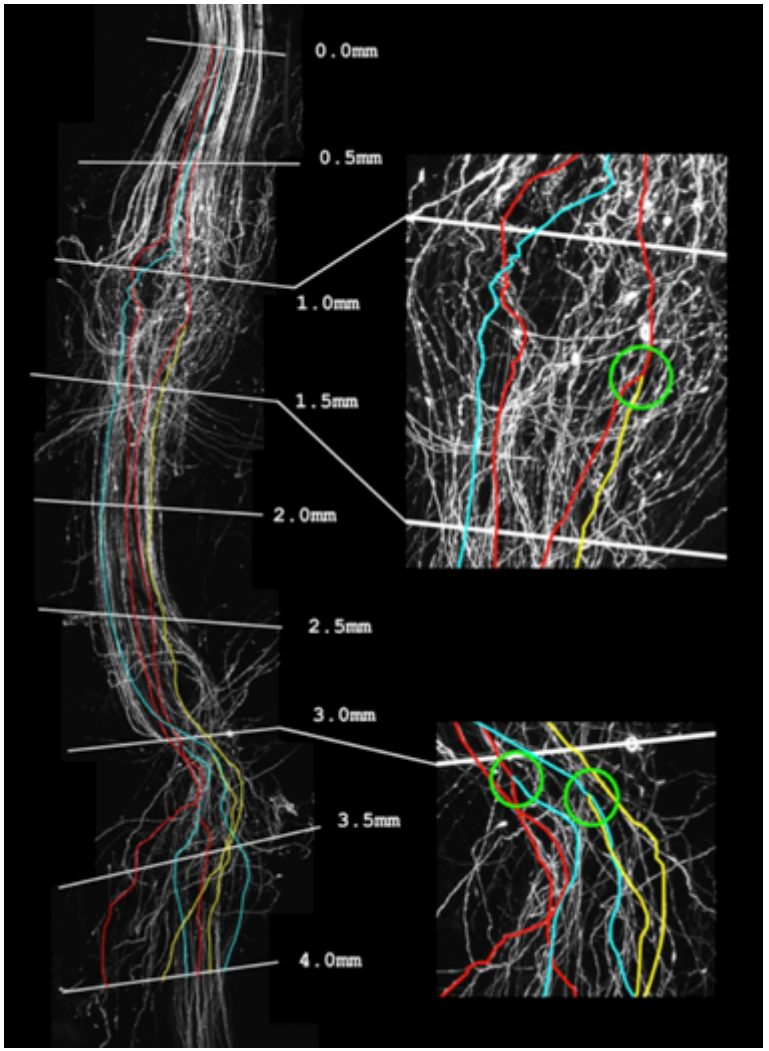
YFP mouse – 3mm common fibular nerve injury model





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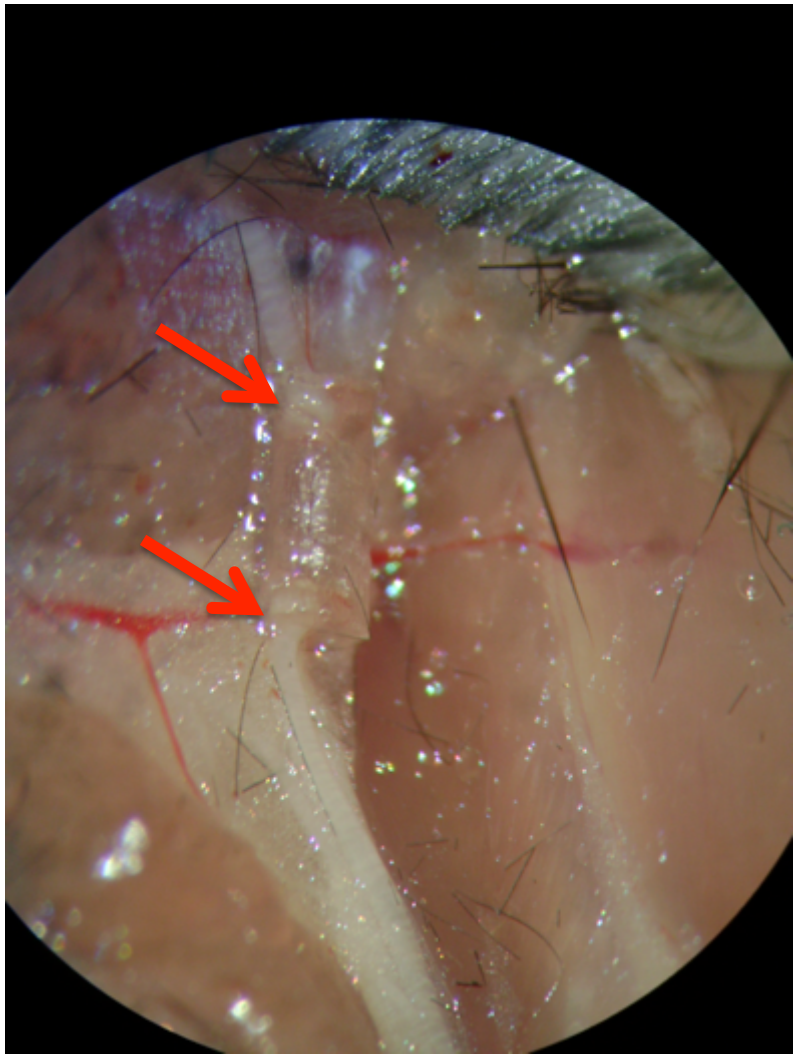

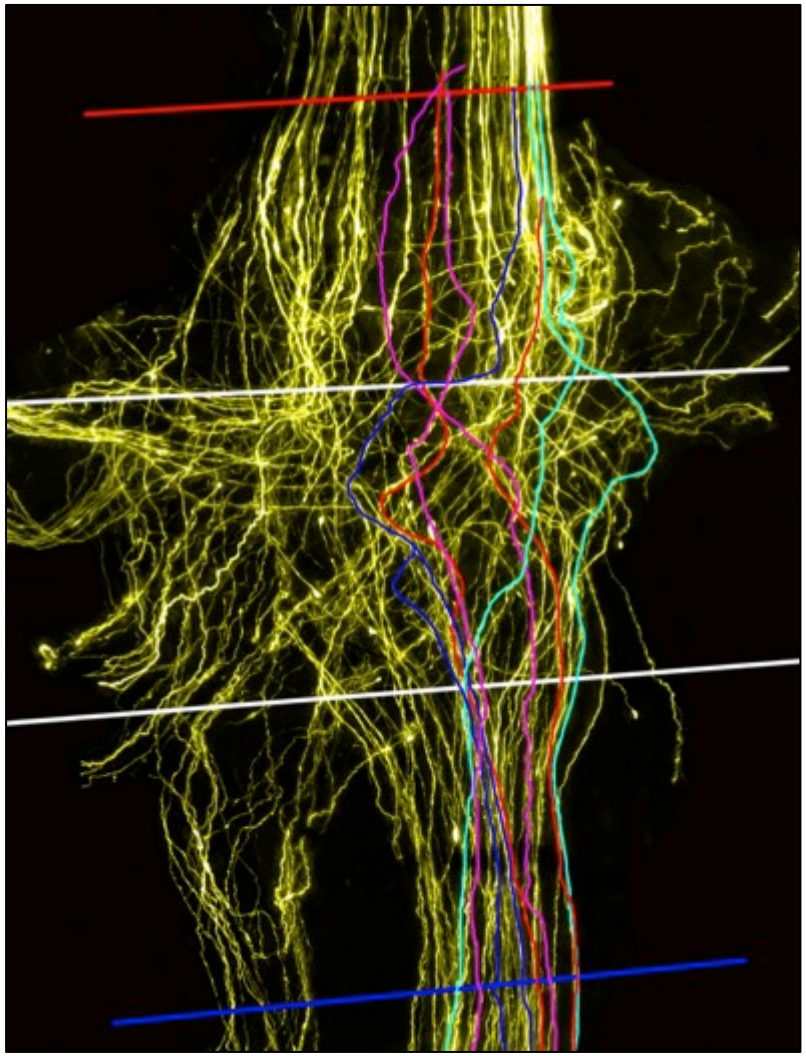

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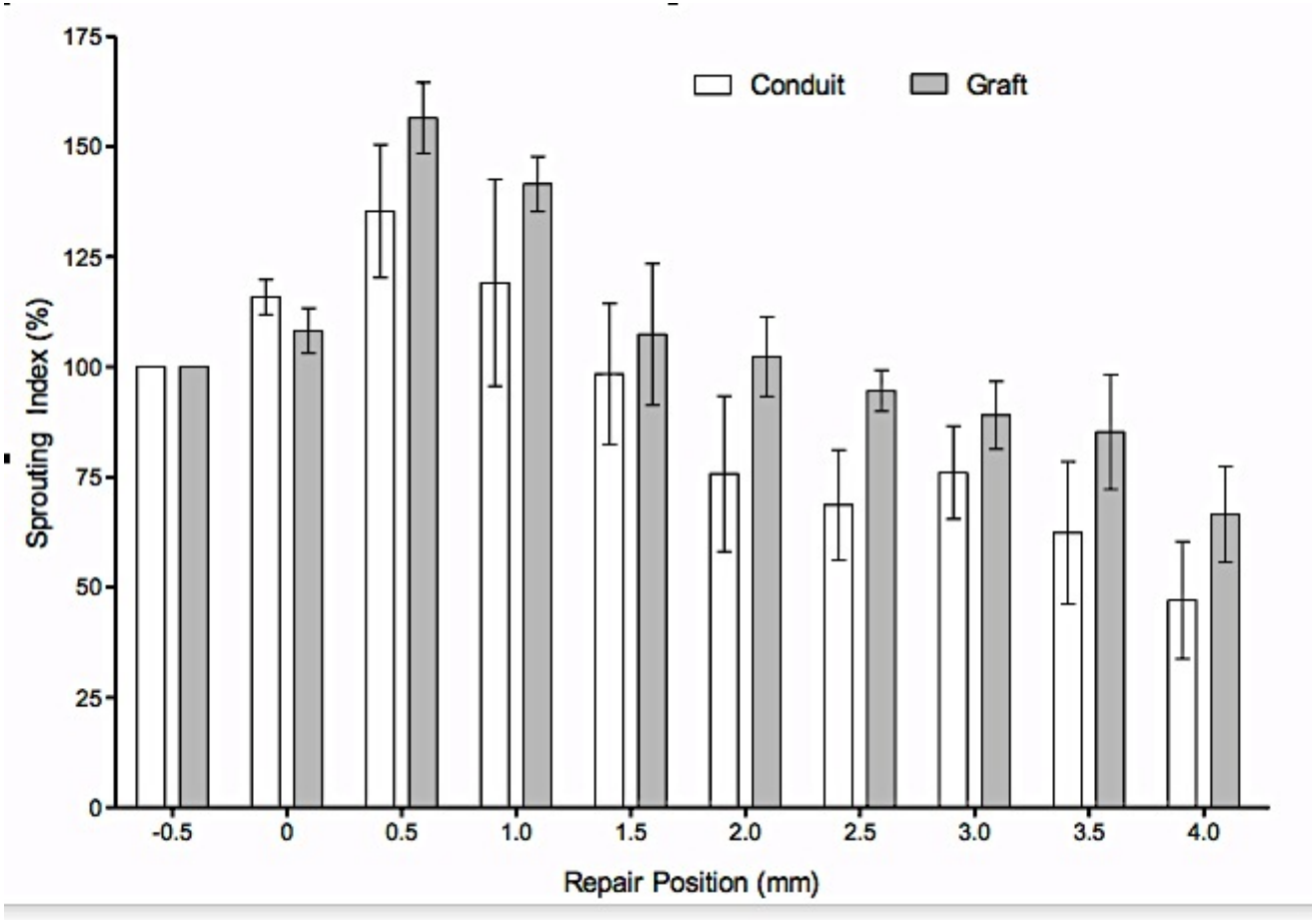
Graft versus nerve conduit repair



Pateman C, Harding A, Glen A, Taylor C, Christmas C, Robinson P, Rimmer S, Boissonade F, Claeyssens F, **Haycock JW**. (2015) Nerve guides manufactured from photocurable polymers to aid peripheral nerve repair. Biomaterials 49, 77–89.

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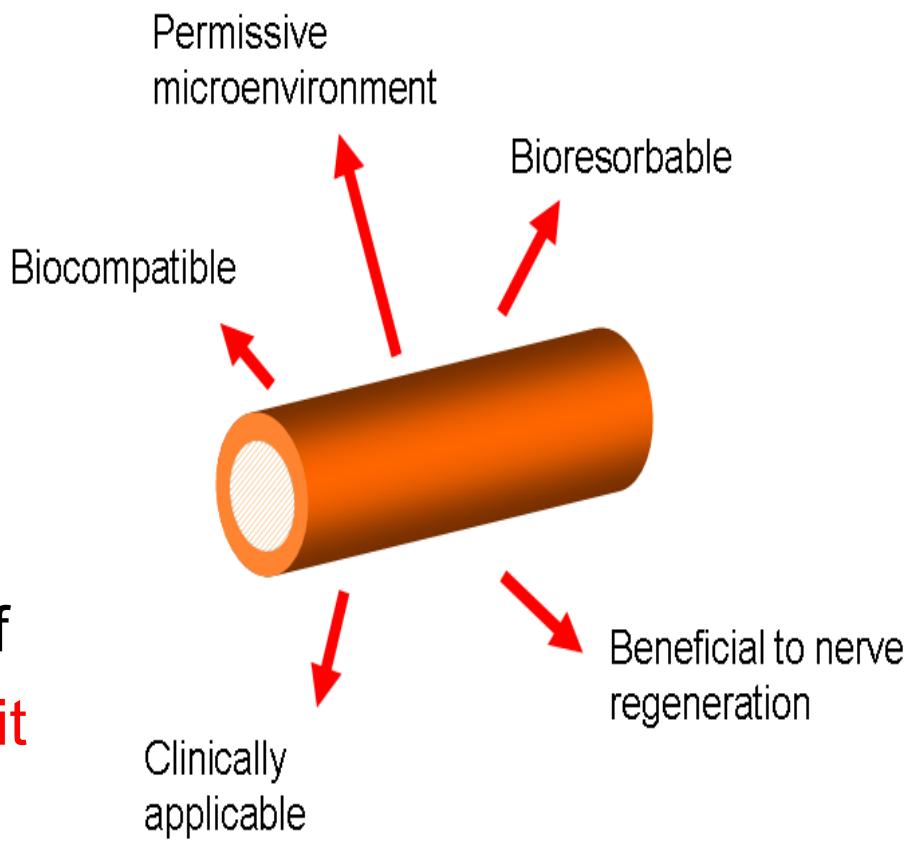

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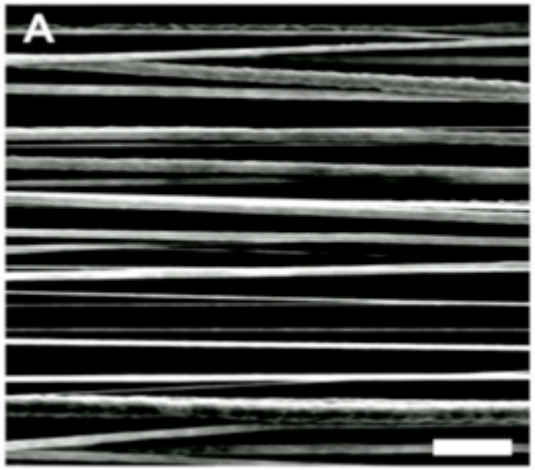
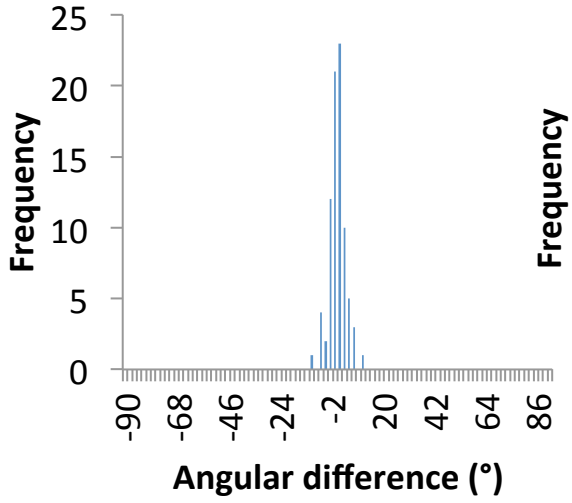
Aligned polycaprolactone fibres

1 µm fibres

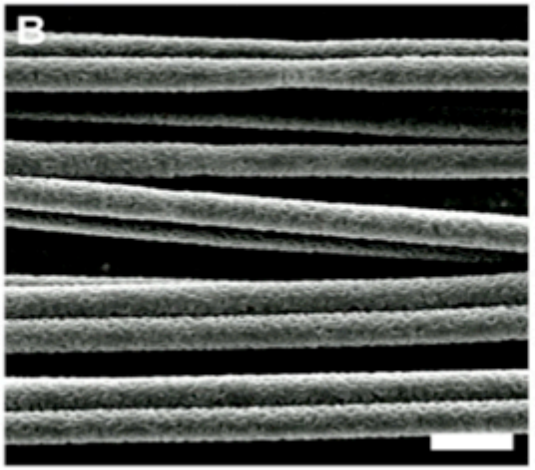
Small fibre

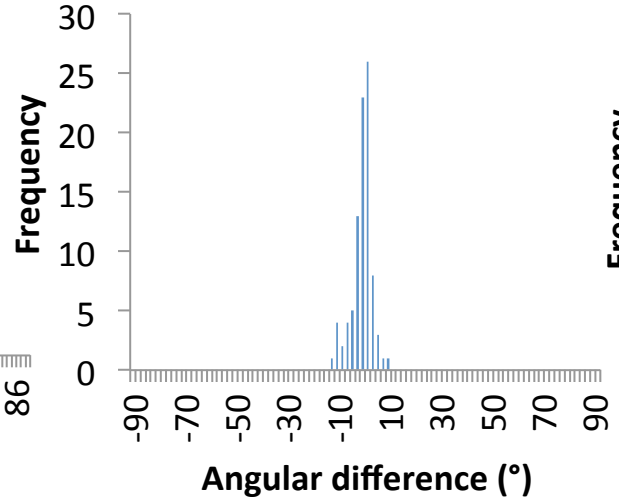




5 µm fibres

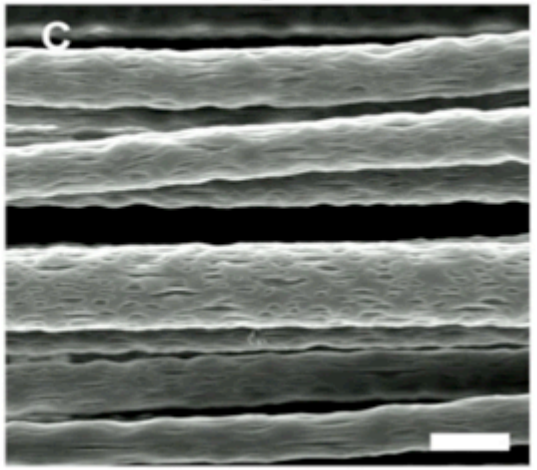
Intermediate fibre

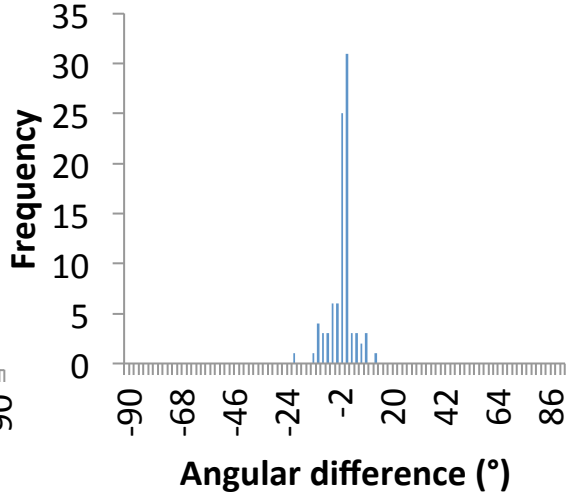




8 µm fibres

Large fibre





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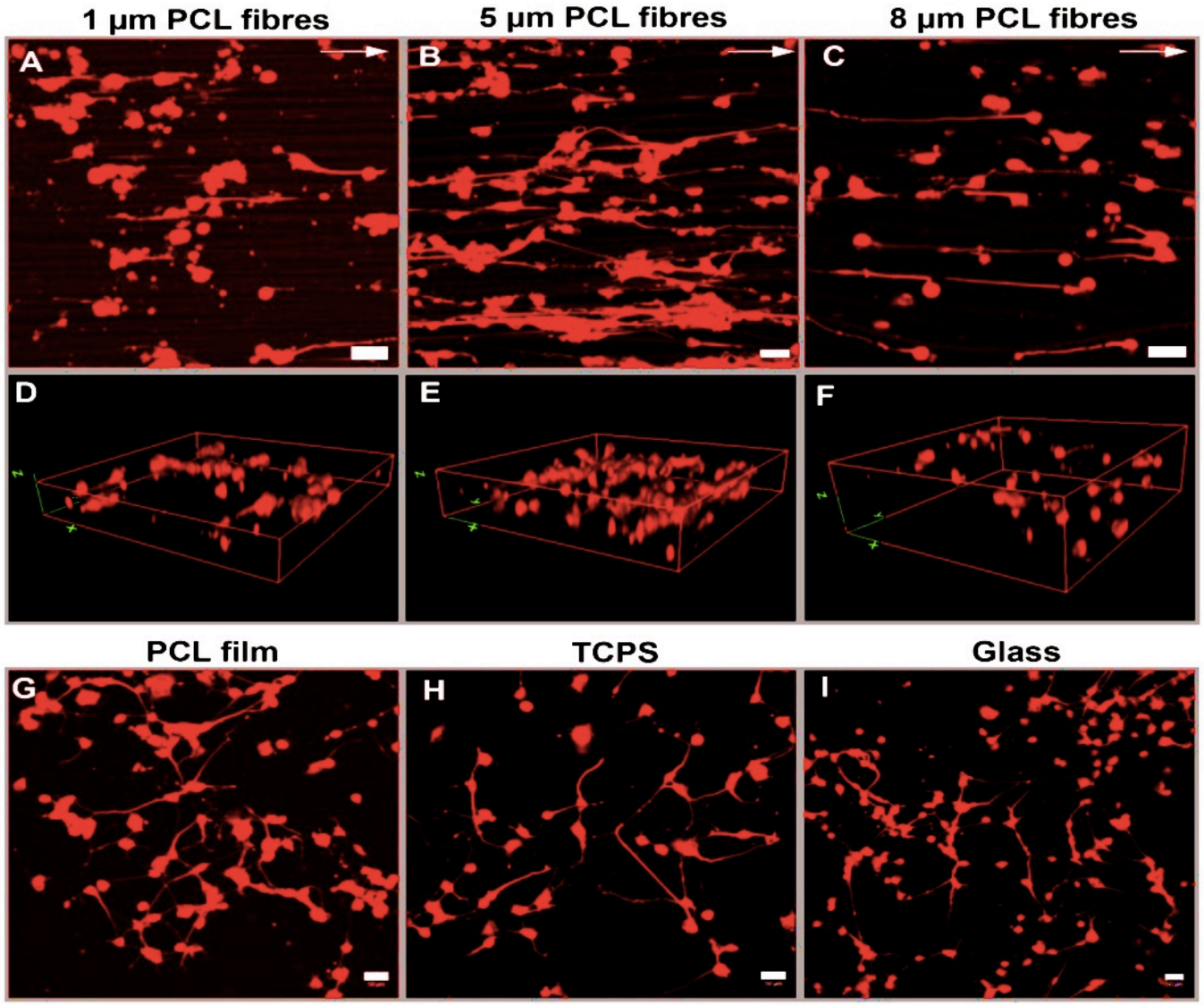
Neuronal cells on aligned polycaprolactone fibres



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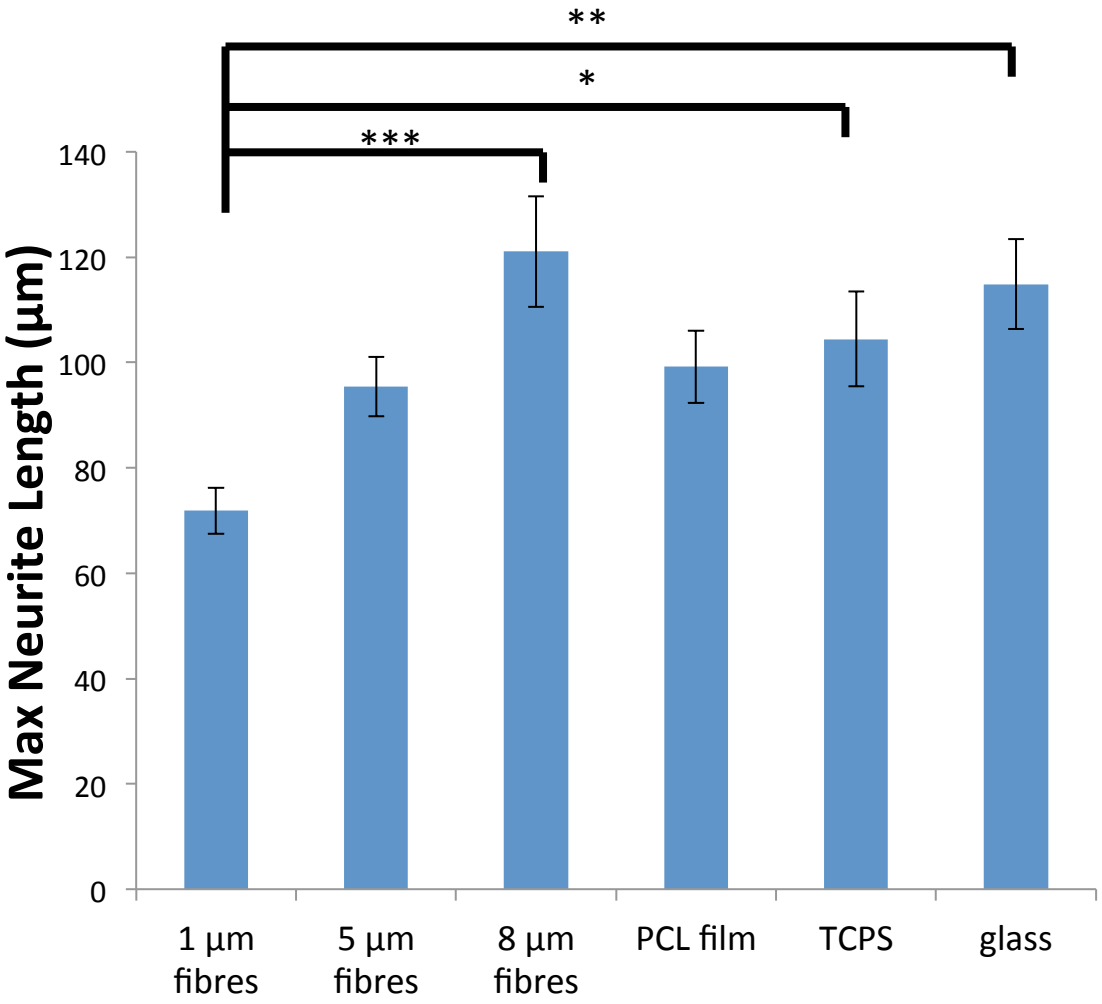
Maximum neurite length



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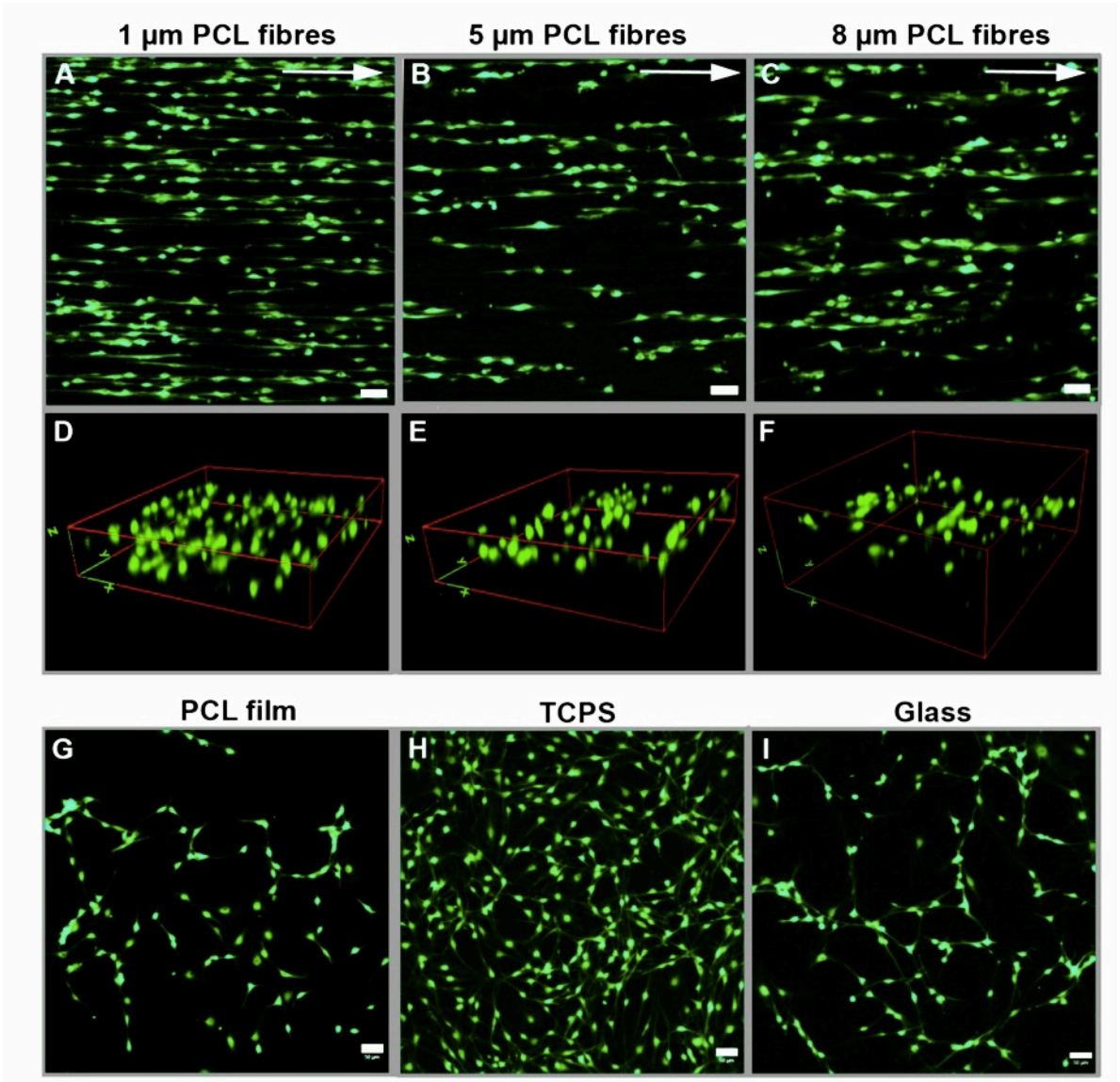
Primary Schwann cells on aligned polycaprolactone fibres



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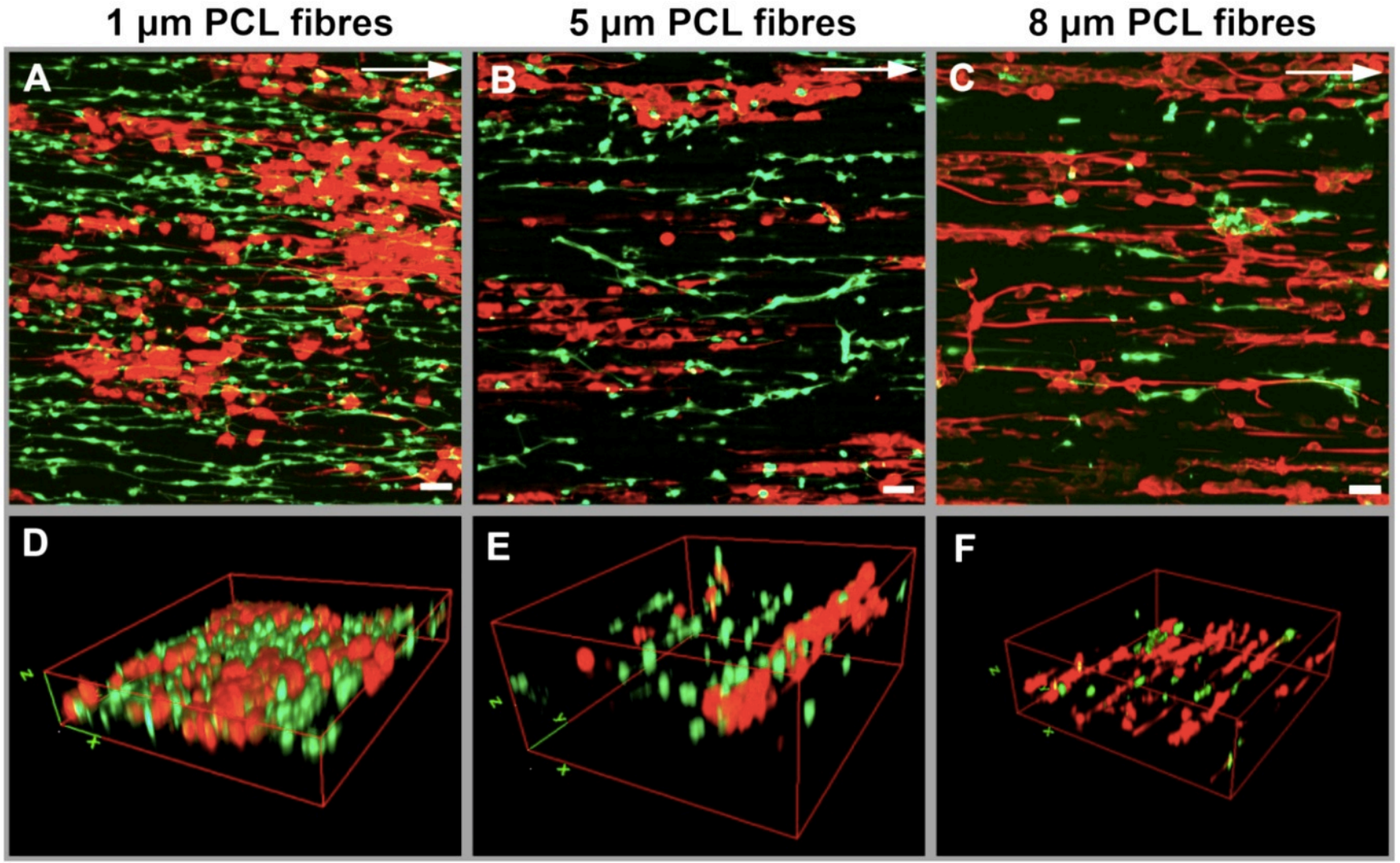
Neuronal cells and primary Schwann cells on aligned polycaprolactone fibres



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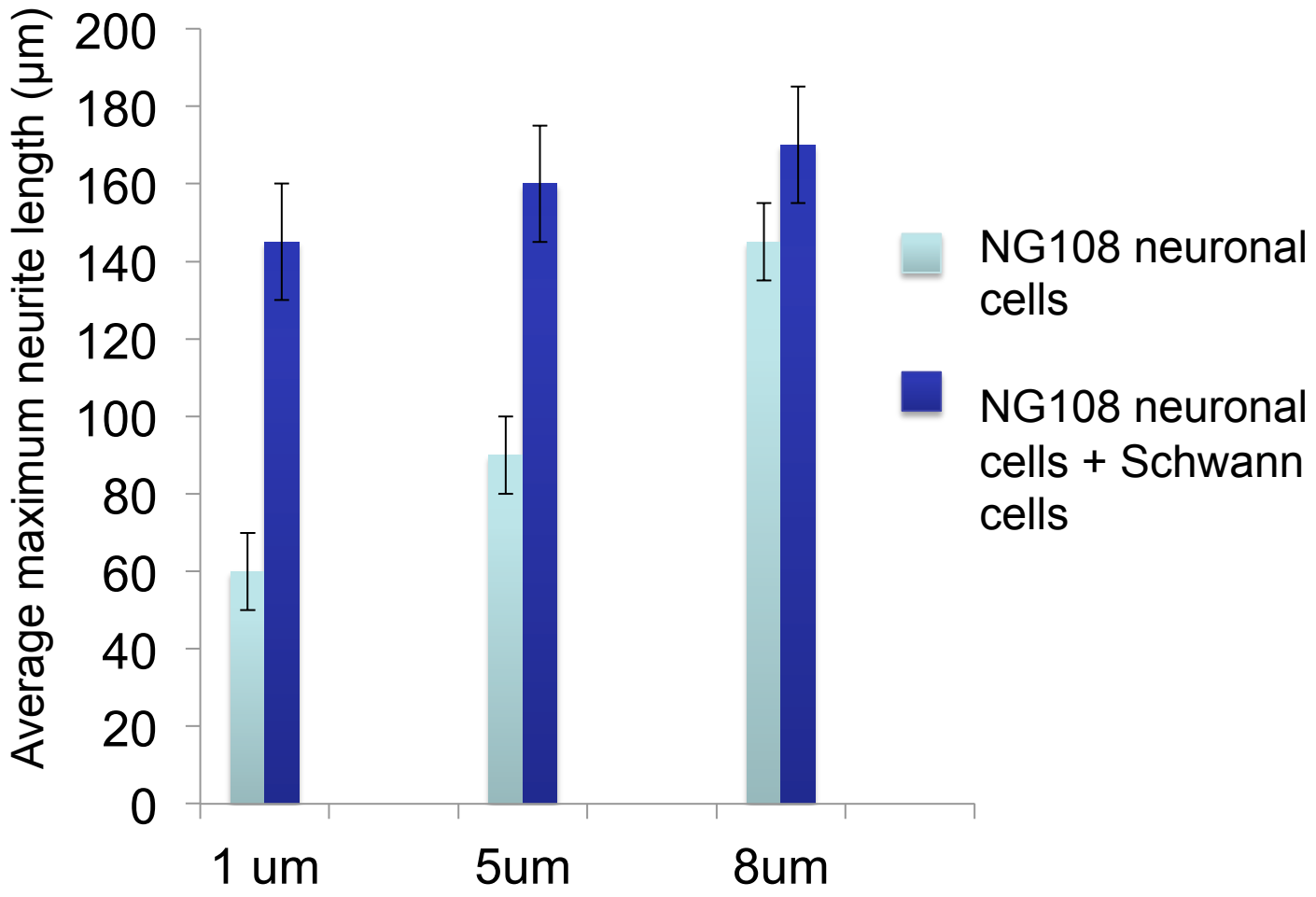

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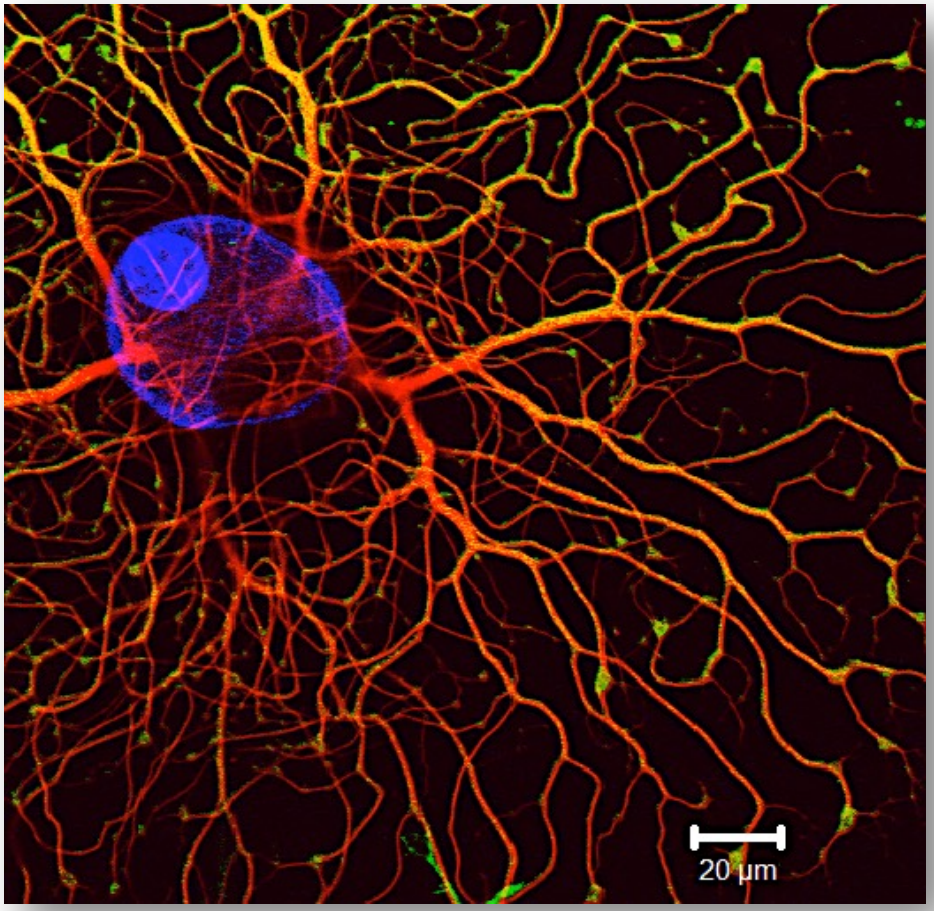
Dorsal Root Ganglion cultures



- On flat culture surfaces DRG neurites form a highly connected but disorganised network

- Nuclei
- β-tubulin-III
- S100β

- Can DRG neurites and Schwann cells be organised to resemble a peripheral nerve?

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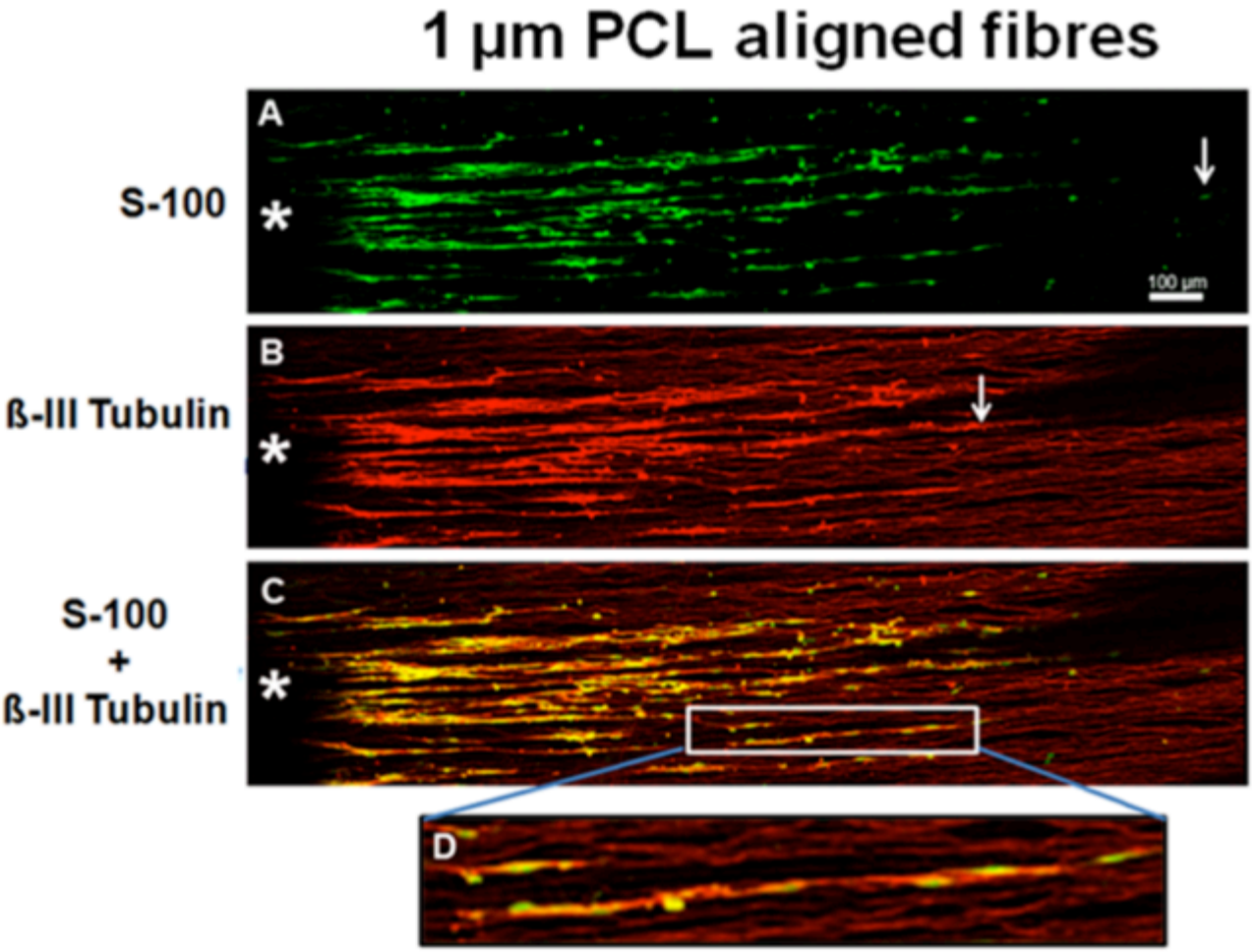
PCL aligned fibre scaffolds for organised growth of DRG neurites and Schwann cells



Daud MFB, Pawar KC, Claeyssens F, Ryan AJ, Haycock JW (2012) An aligned 3D neuronal glial co-culture model for peripheral nerve studies. ***Biomaterials*** 33(25) 5901-5913.

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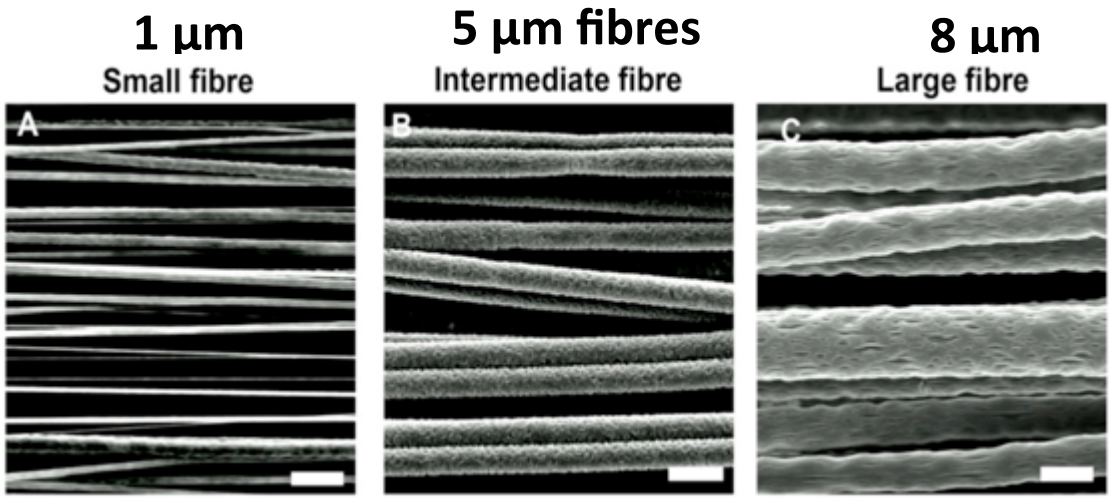
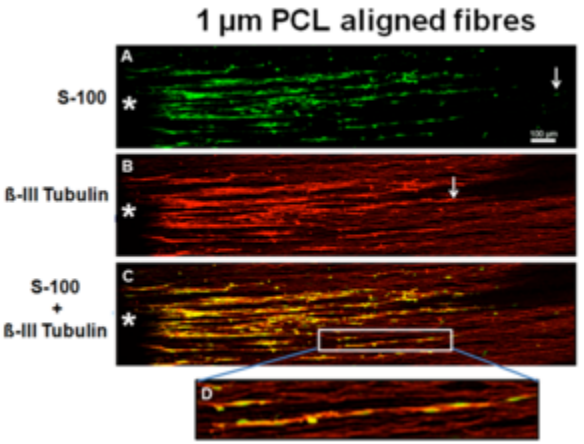
PCL aligned fibre scaffolds for organised growth of DRG neurites and Schwann cells





1. Nerve guides
2. 3D *in vitro* model of peripheral nerve

Daud MFB, Pawar KC, Claeyssens F, Ryan AJ, Haycock JW (2012) An aligned 3D neuronal glial co-culture model for peripheral nerve studies. ***Biomaterials*** 33(25) 5901-5913.

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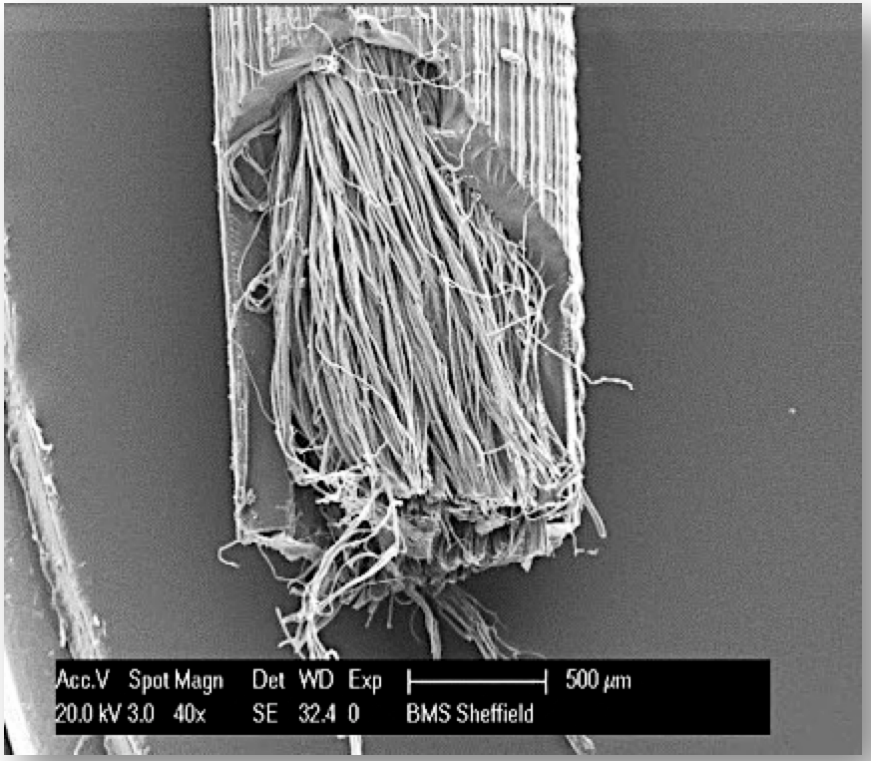
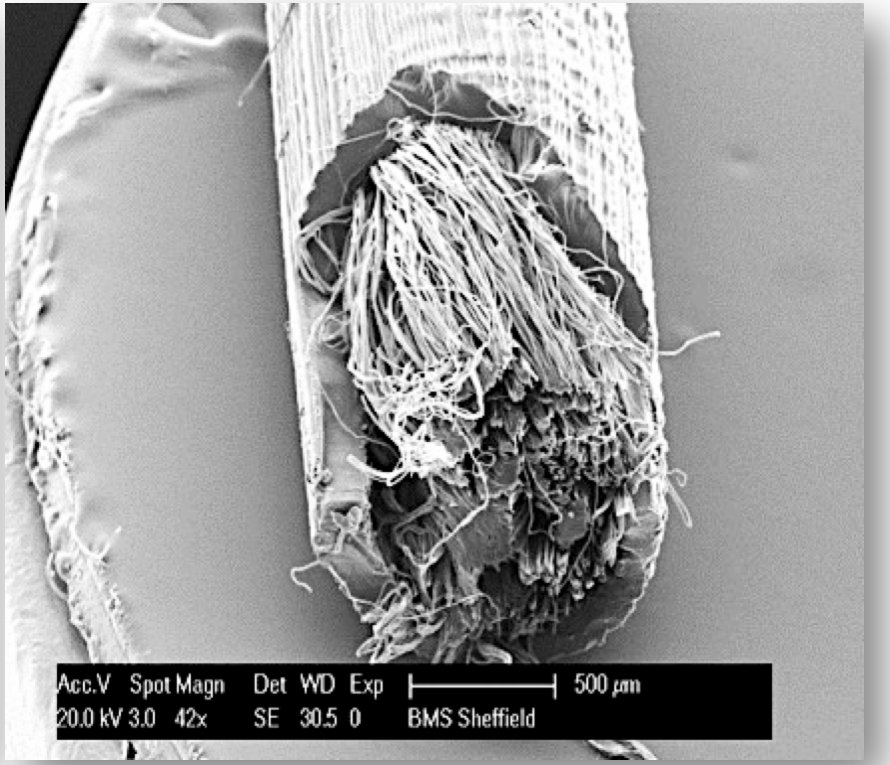
Photocurable poly(ethyleneglycol) conduit + poly(caprolactone) fibres





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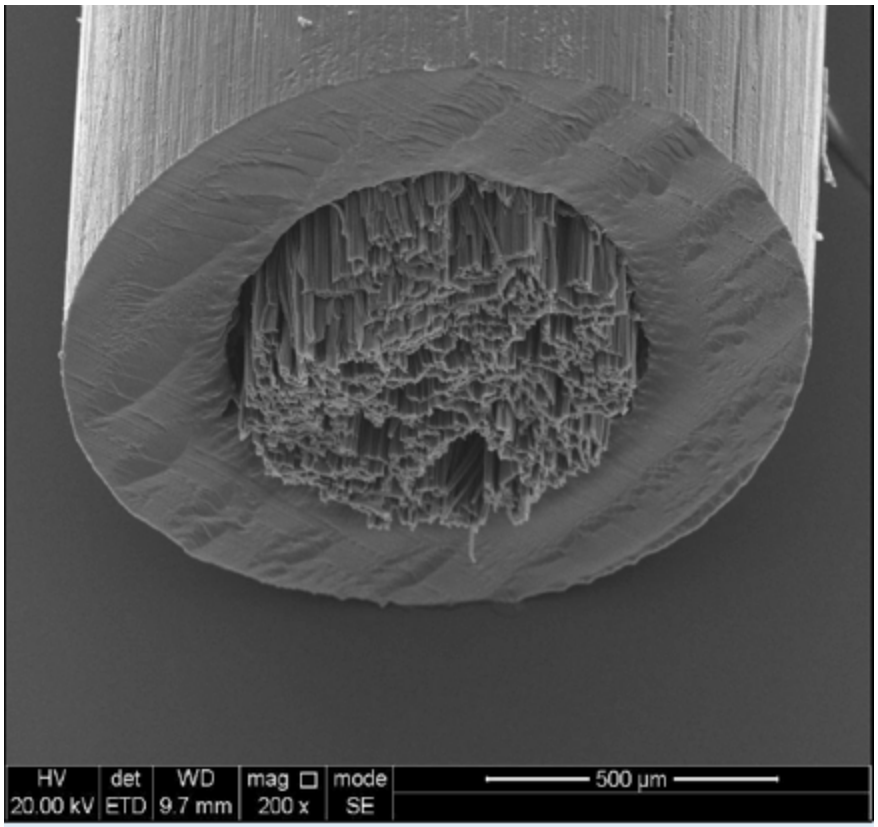
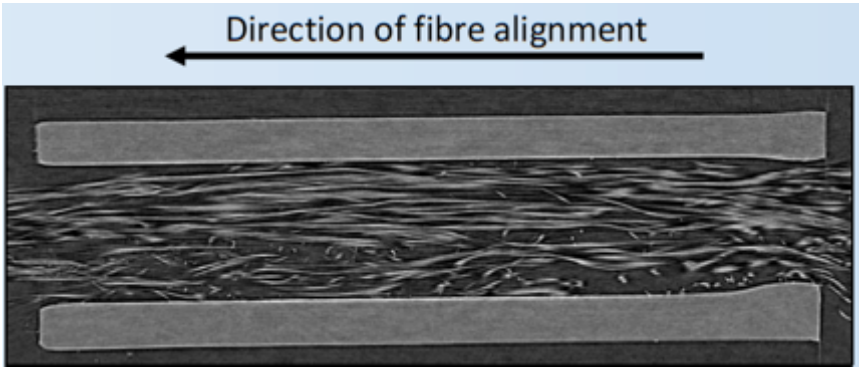
Photocurable poly(caprolactone) conduit + poly(caprolactone) fibres

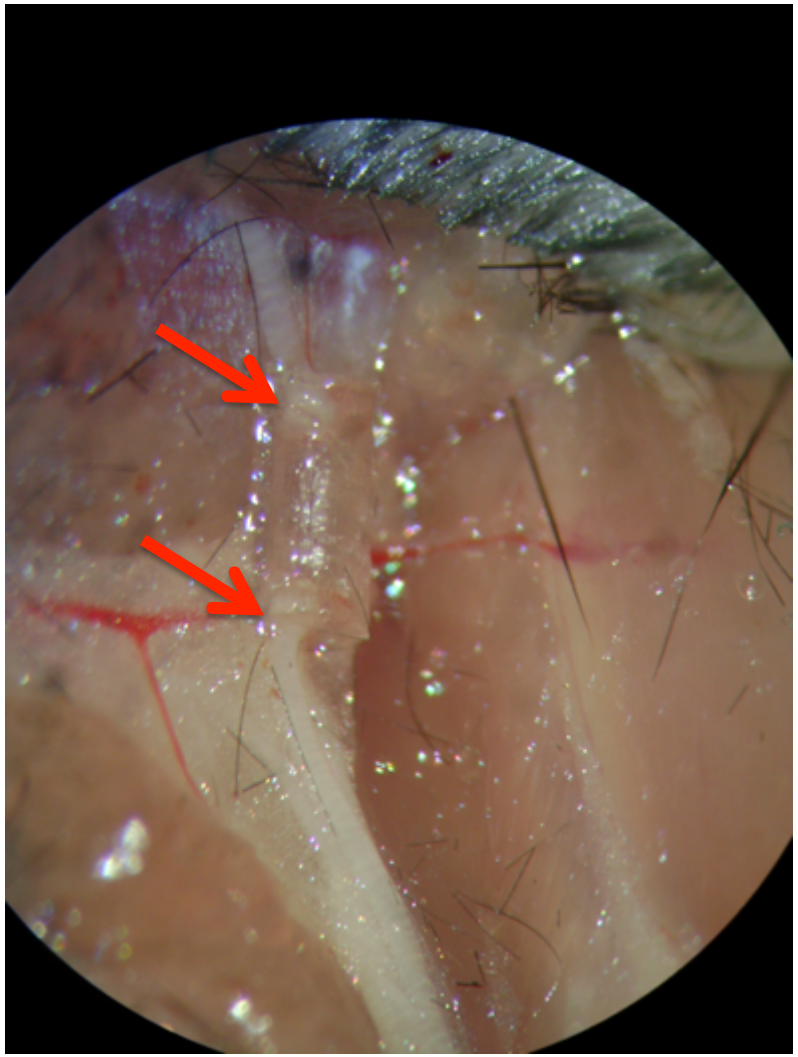


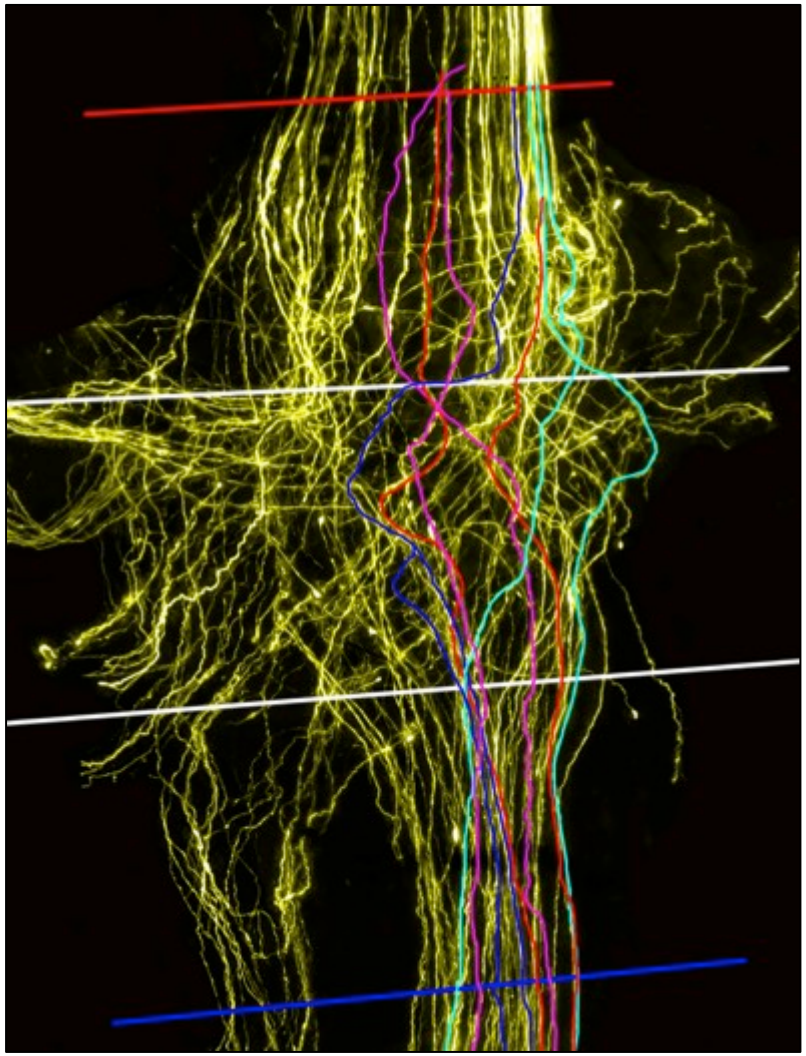


Mr Jonathon Field, PhD student (EPSRC), Materials Science & Engineering, University of Sheffield

The University Of Sheffield.

YFP mouse – 3mm common fibular nerve injury model





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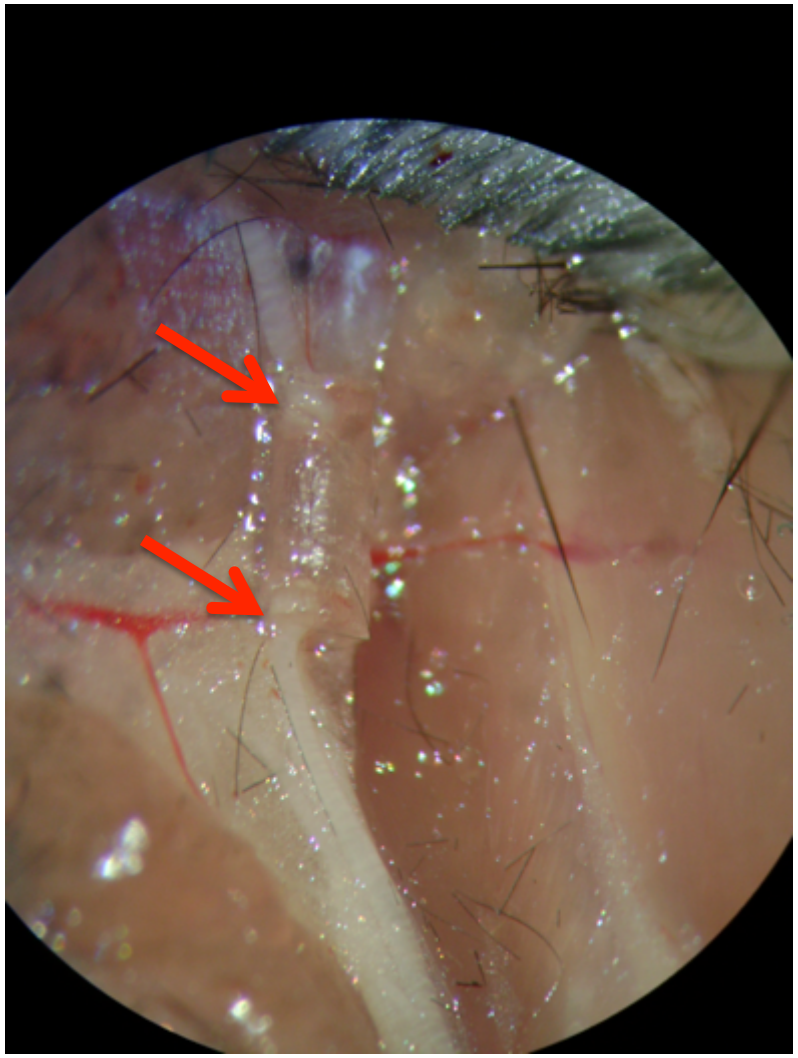
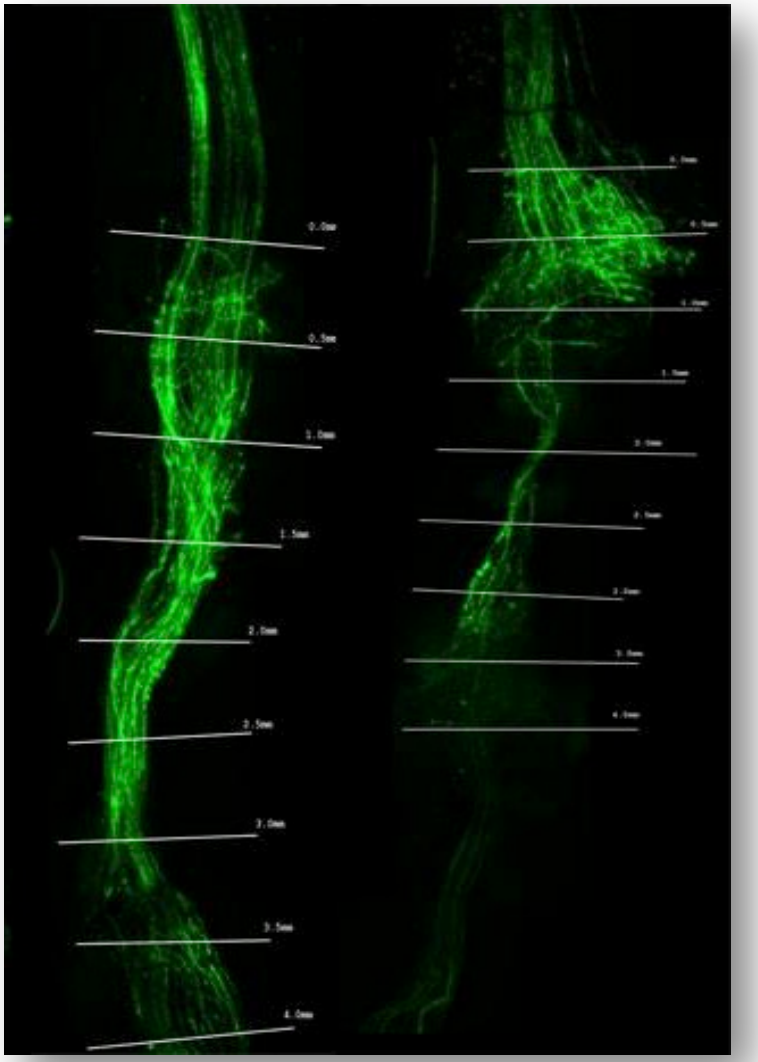
Photocurable poly(ethyleneglycol) conduit + poly(caprolactone) fibres





500 fibres 1000 fibres

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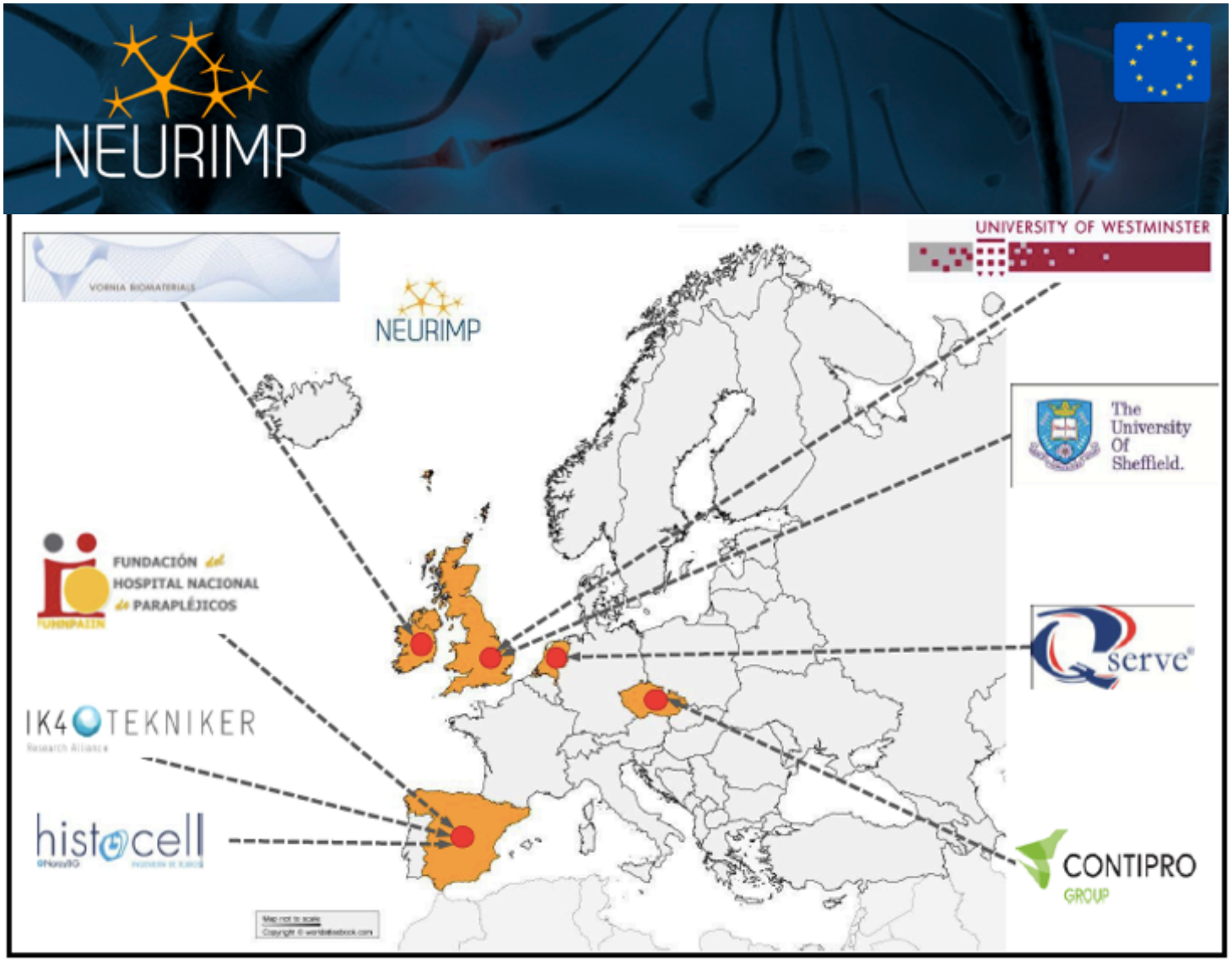
Future strategies for bioengineering peripheral nerve

FP7 – NEURIMP - €4.6 million – 2014 - 2018



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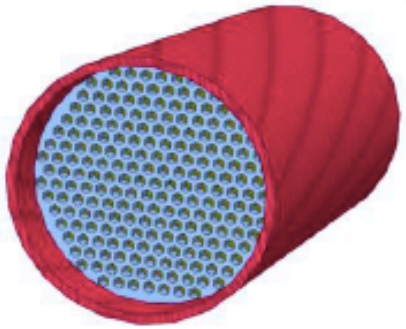
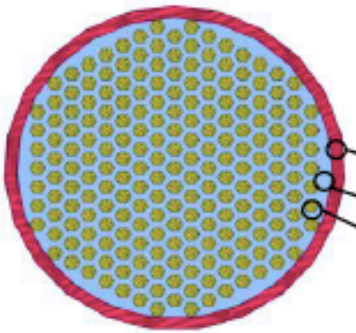
Nerve Repair – New materials and processing techniques

a)





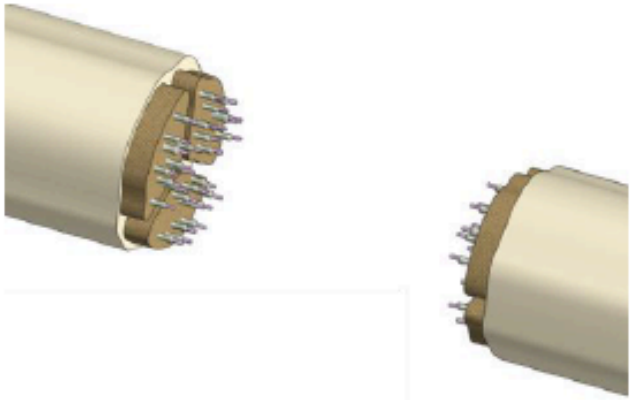
Different location and role of materials depending on their nature:

Material 1 (stiff): external tube

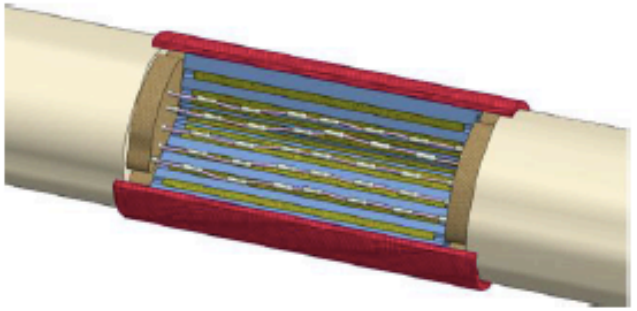
Material 2 (stiff): internal skeleton

Material 3 (soft): inside microchannels

b)



c)



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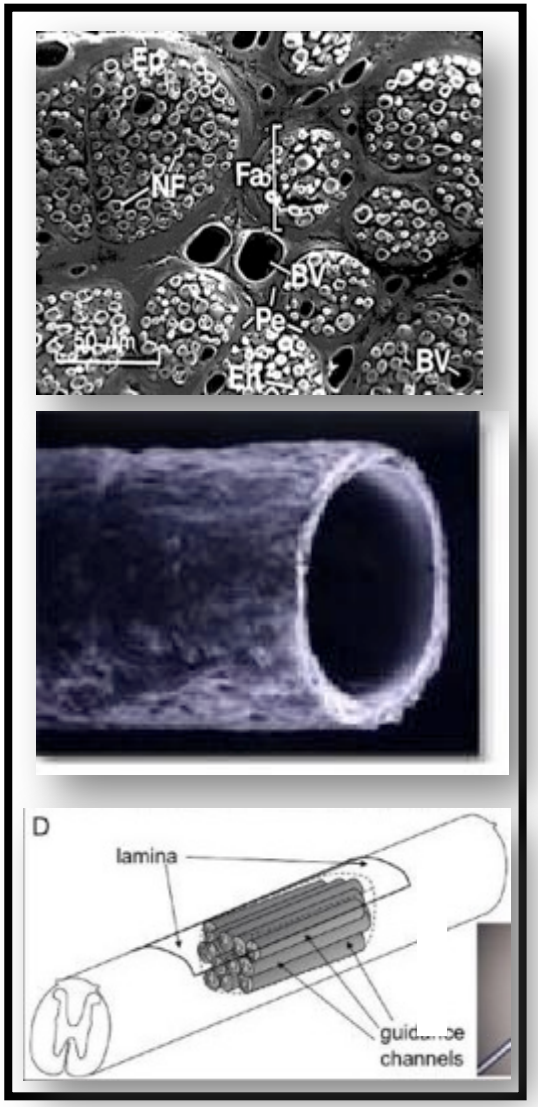
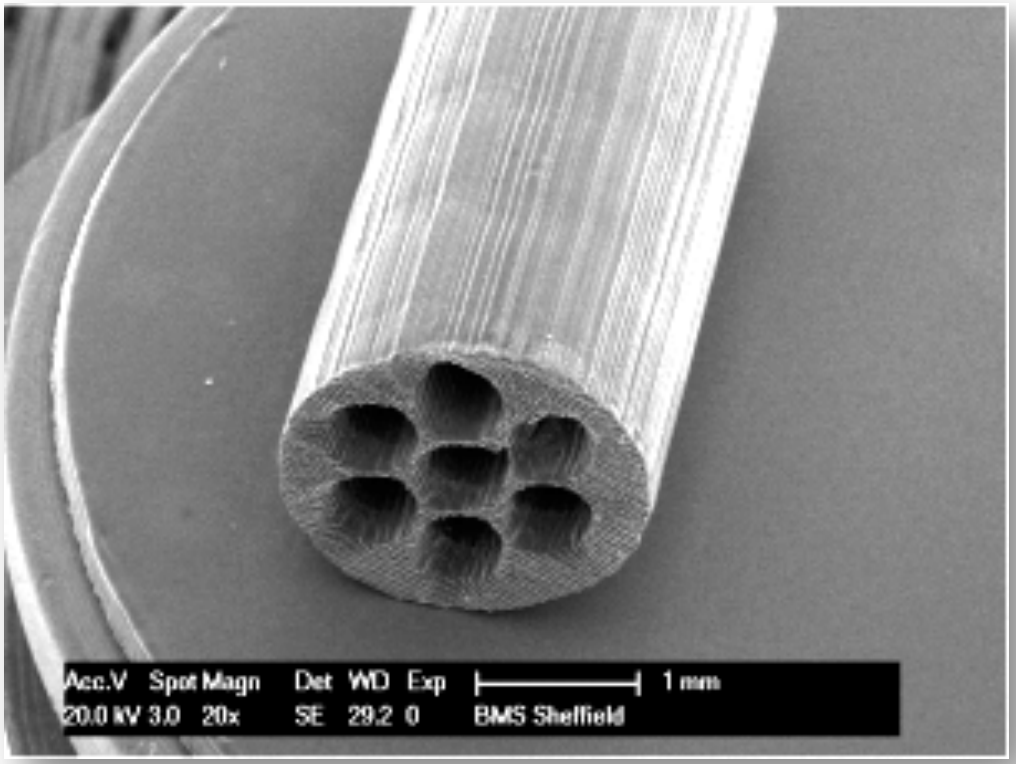
Nerve guidance conduits



1. Manufacture of NGCs from PEG, PLA or PCL➔ Polyhydroxyalkoanates

2. Incorporate internal structure within the tube to improve regeneration



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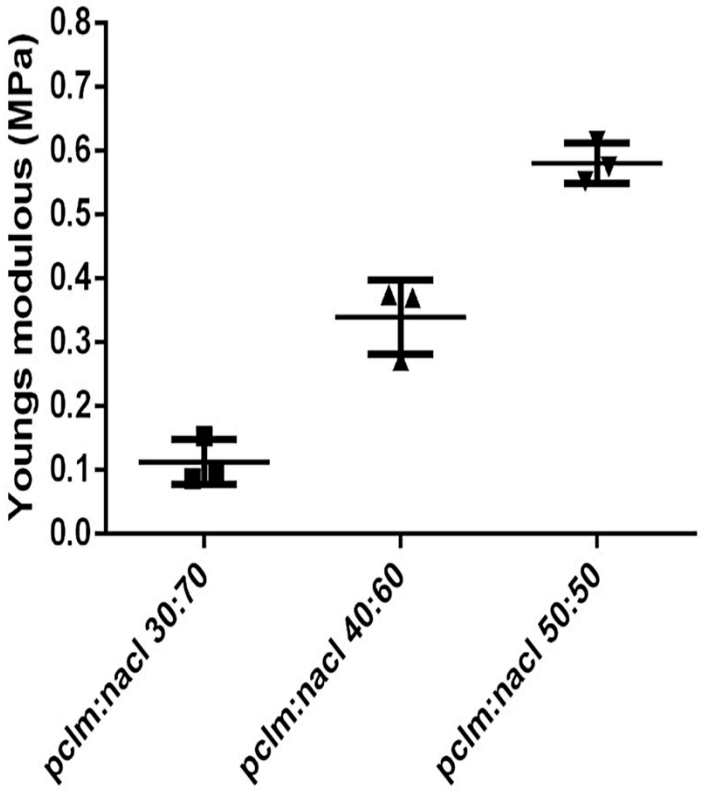
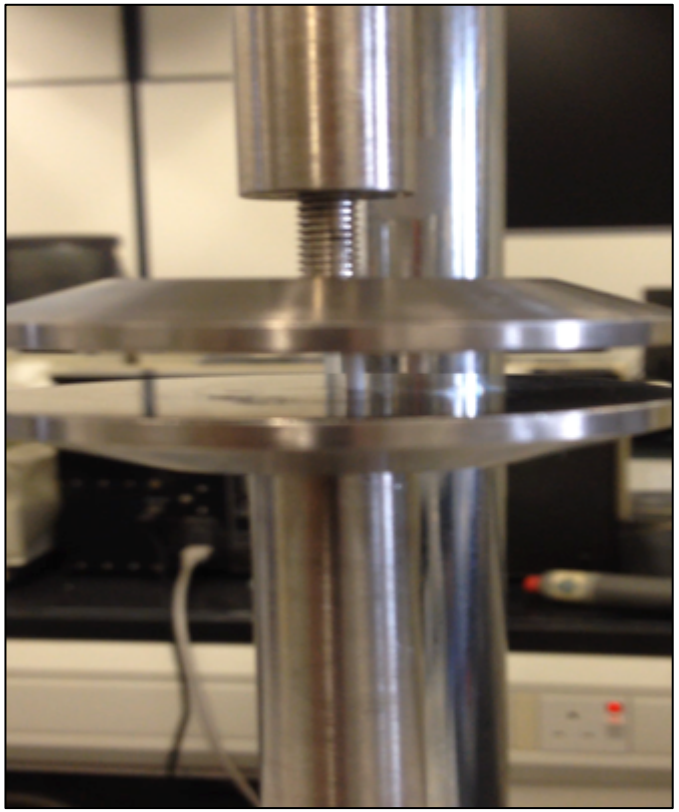
Compression testing of UV casted channelled NGCs

Similar to rat sciatic nerve Young's modulus





Borschel et al. (2003) = rat sciatic nerve Young's Modulus of 0.58 ± 0.015 MPa

PCLm/NaCl 50:50 = Young's modulus of 0.58 ± 0.016 MPa

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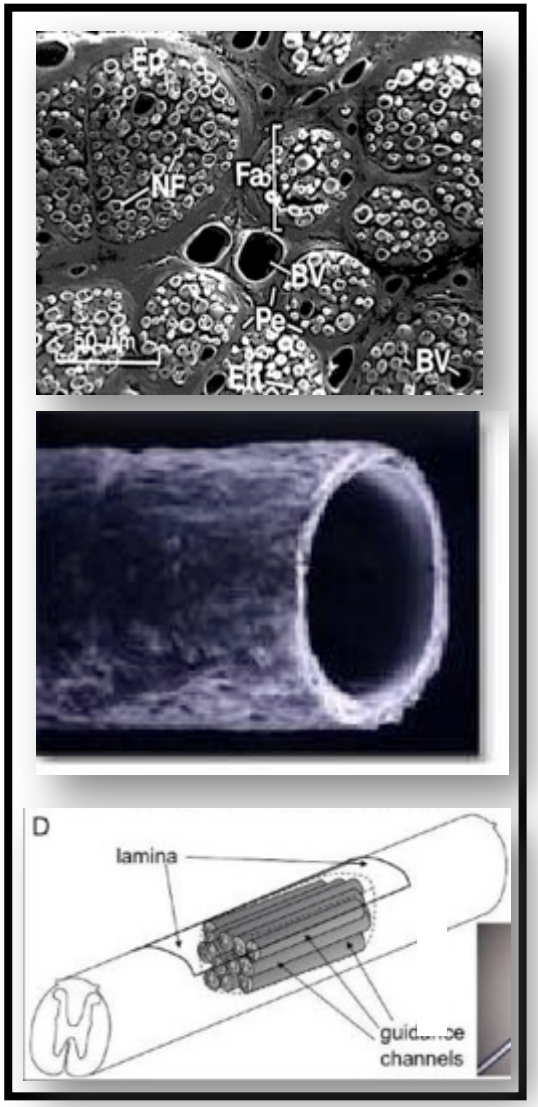
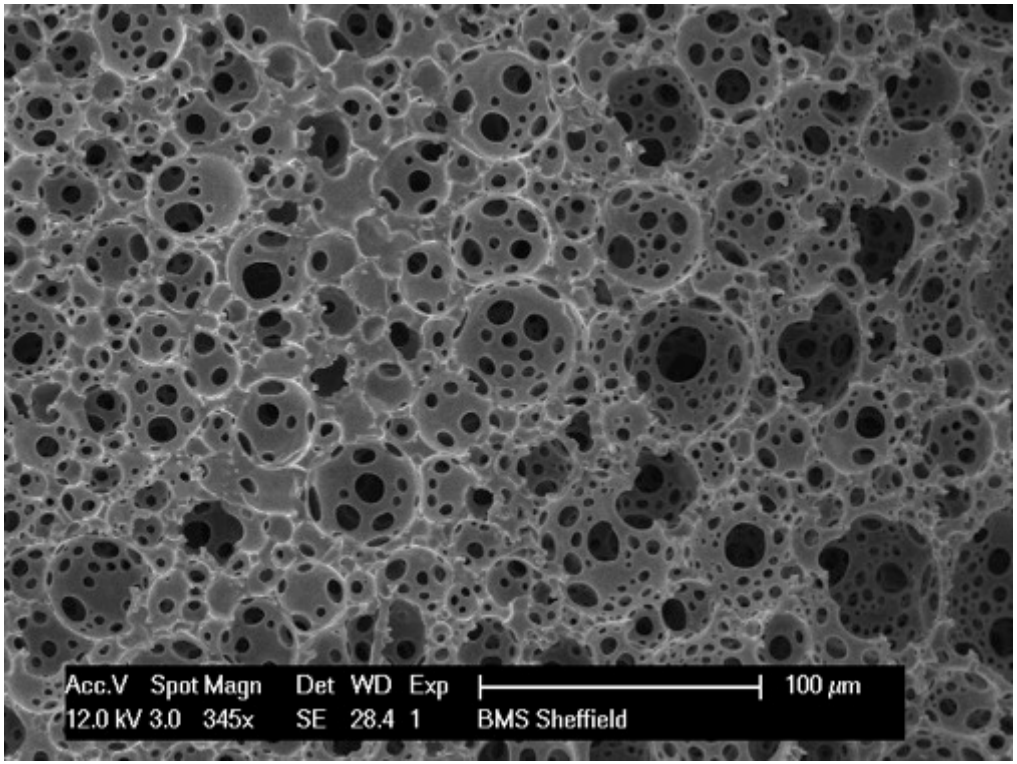
Nerve guidance conduits PolyHIPE



1. Manufacture of NGCs from PCL

2. Incorporate porosity by direct write



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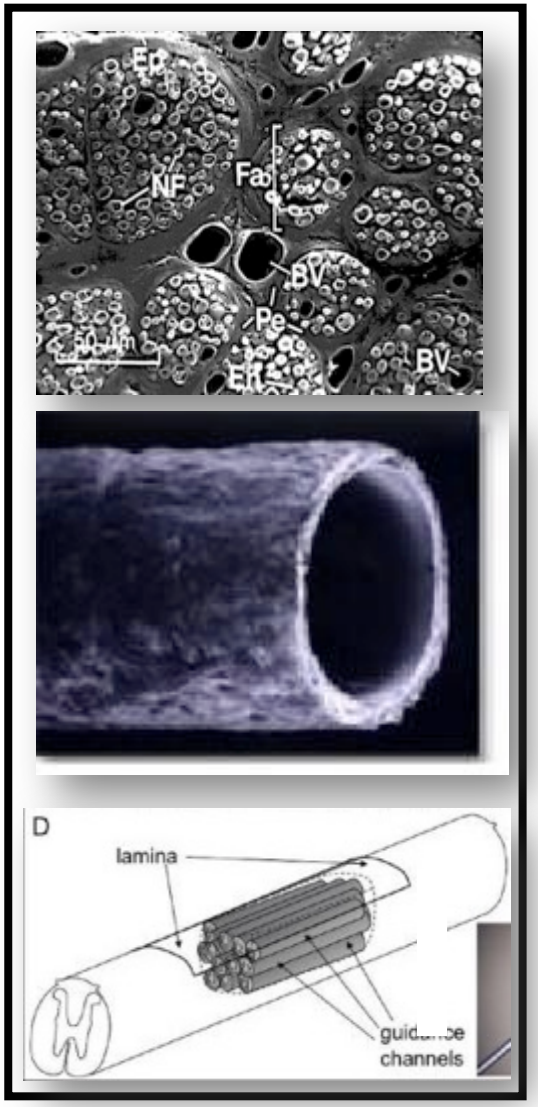
Nerve guidance conduits PolyHIPE



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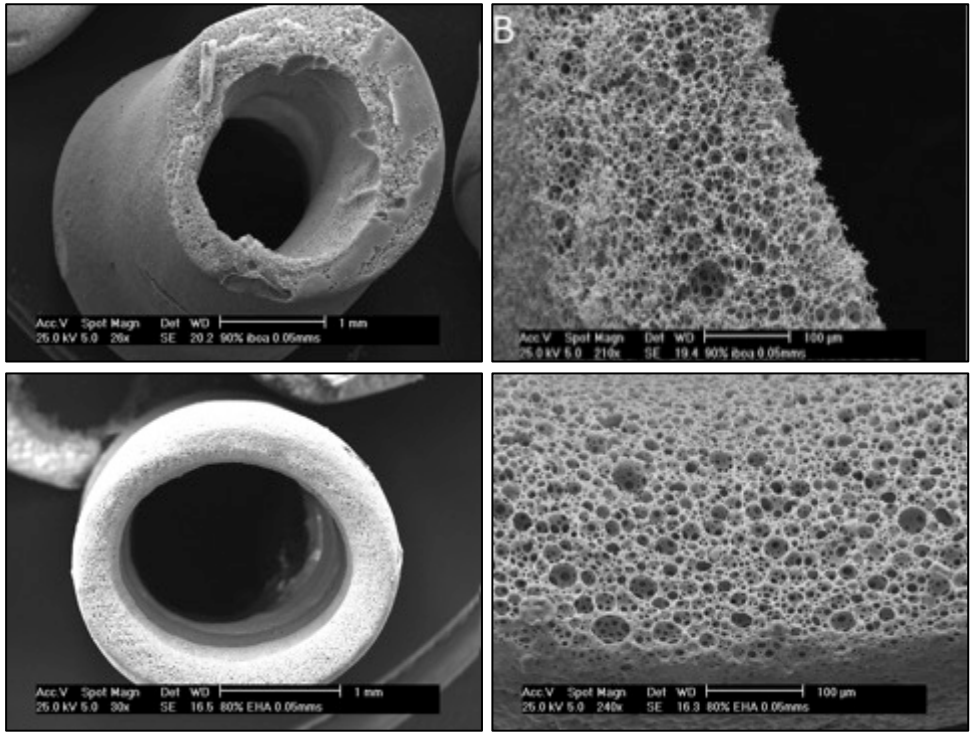

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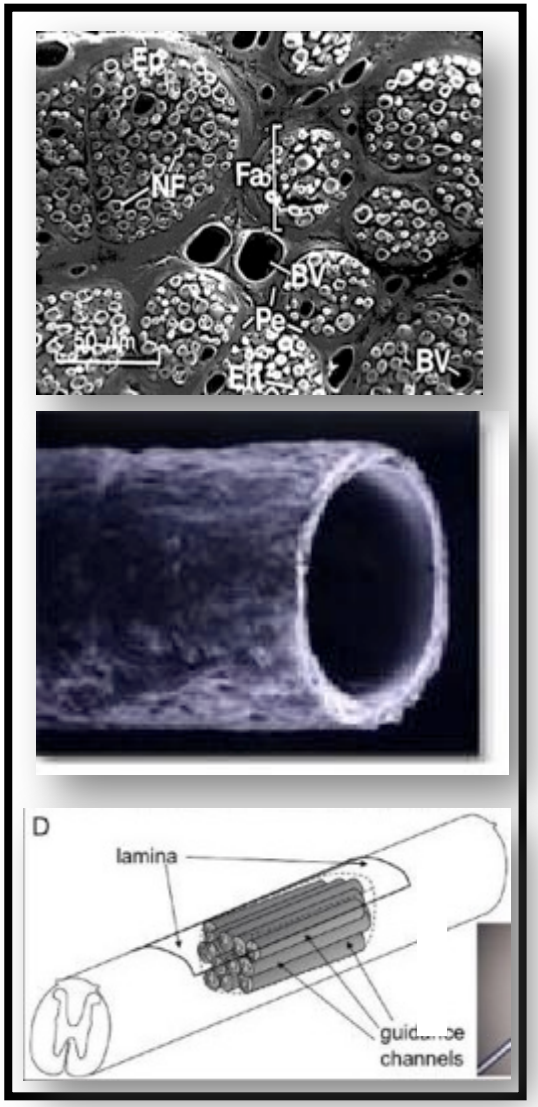
Nerve guidance conduits PolyHIPE



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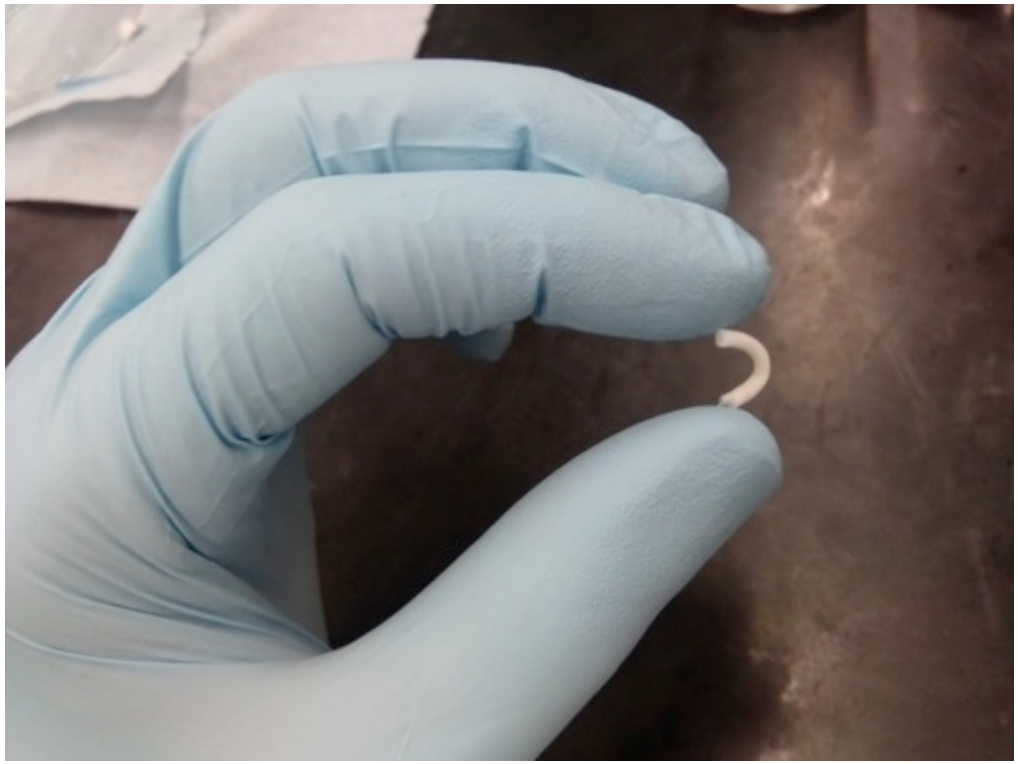

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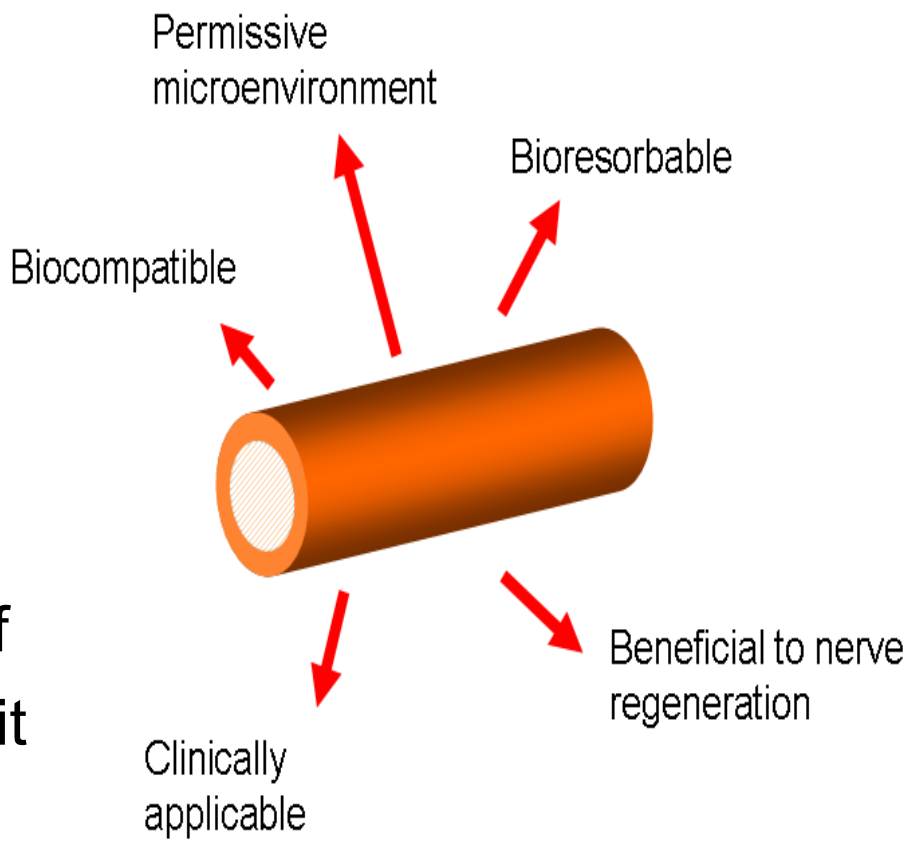
Present strategies for bioengineering peripheral nerve

- To increase regeneration distance
- To improve extent and effectiveness of reinnervation
- Involves a combination of
  1) Nerve guidance conduit
  2) Schwann cells



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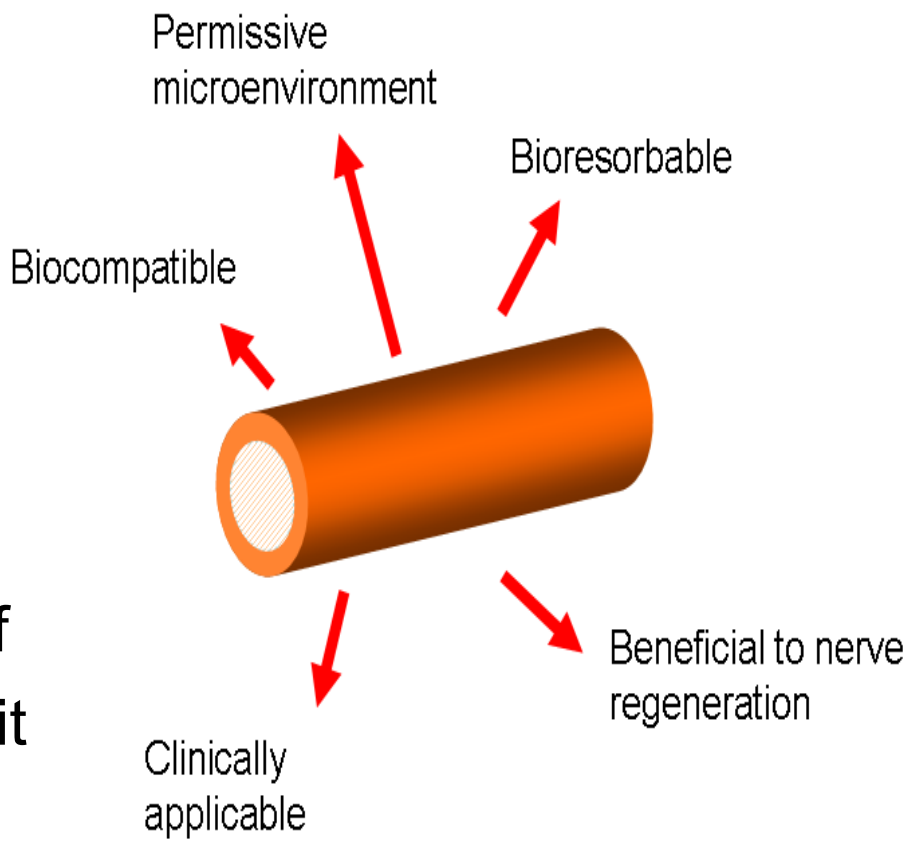

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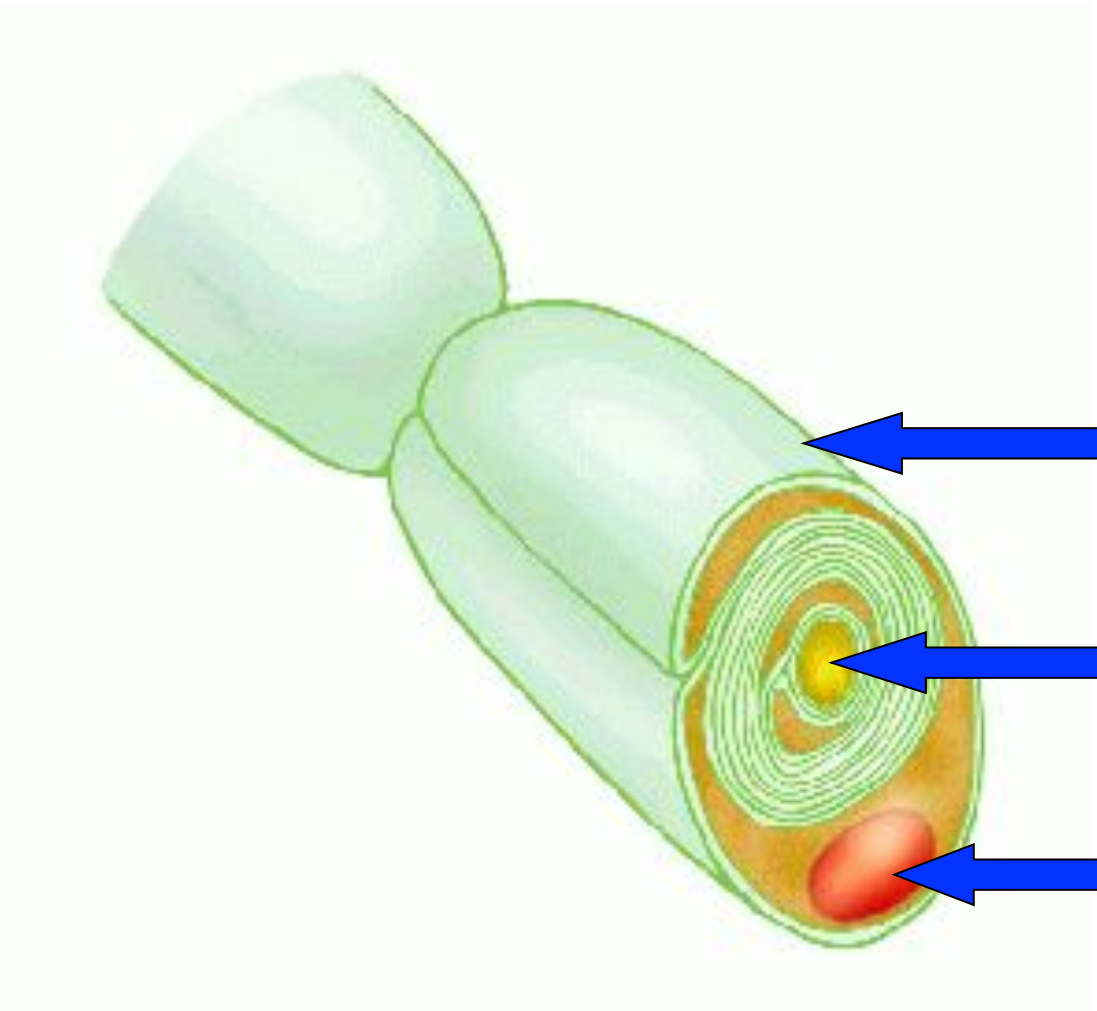
Schwann cells



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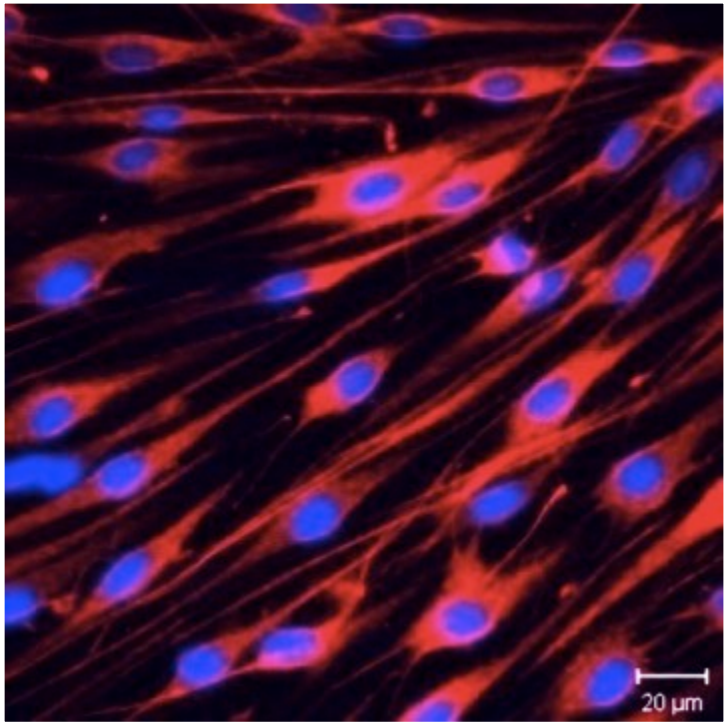
Schwann cells

Role of Schwann cells

- Provide structural support for nerve fibres
- Produce growth factors
- Essential for successful nerve regeneration



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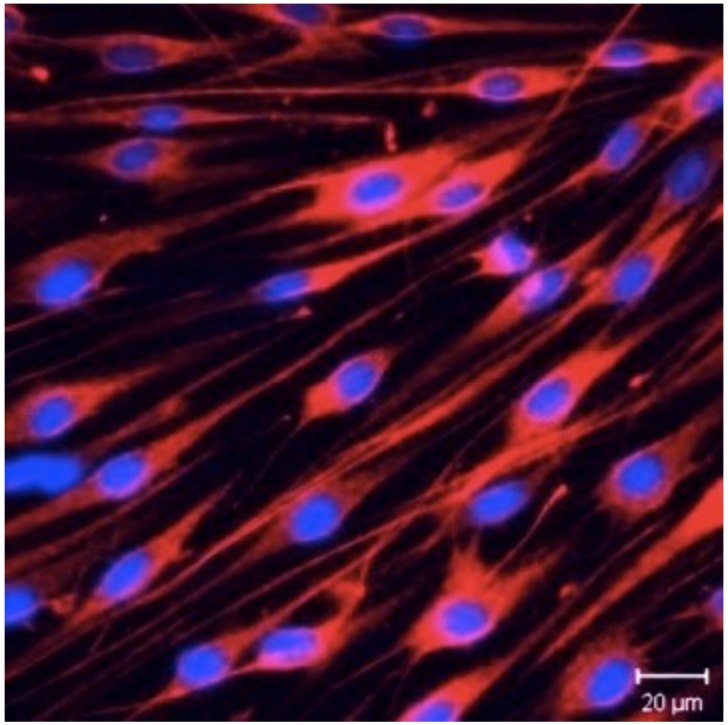
Schwann cells

Role of Schwann cells

- Extremely difficult to culture
- Heavily contaminated with fibroblasts
- Require 3-4 months + large nerve samples for sufficient numbers clinically



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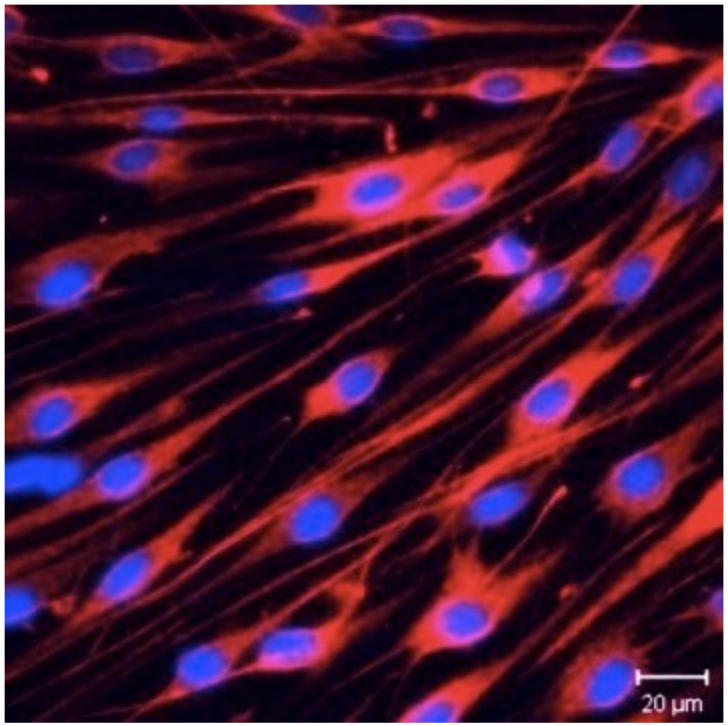
Schwann cells

Role of Schwann cells

- A simple method for culture needed
- **Hypothesis** – Is there a differential expression of *D-amino acid oxidase* between Schwann and fibroblasts?
- Selective culture in D-valine medium



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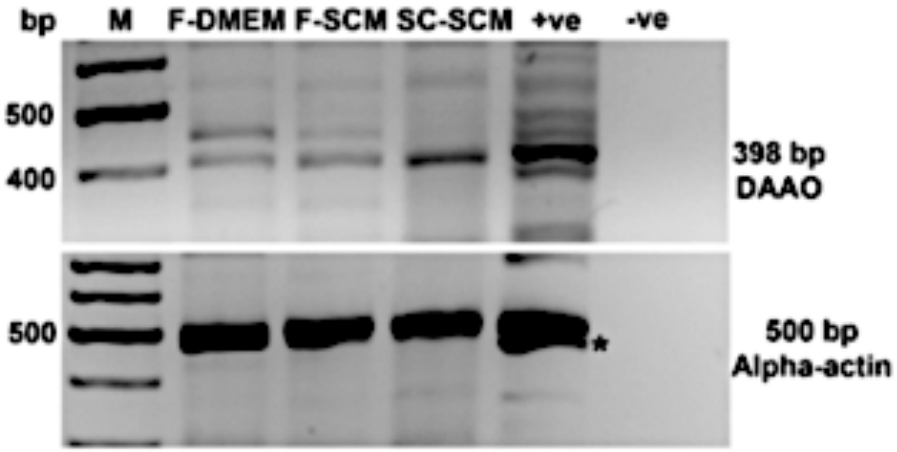
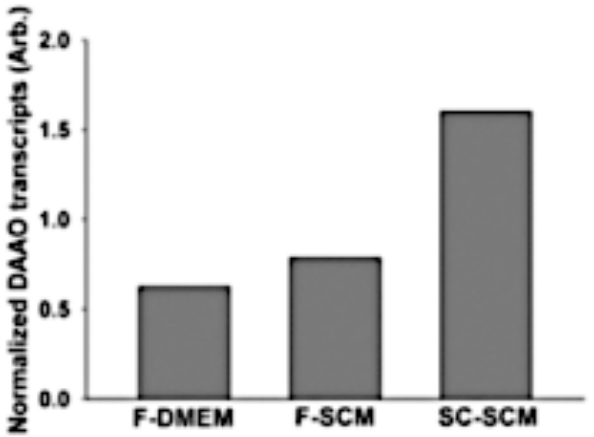
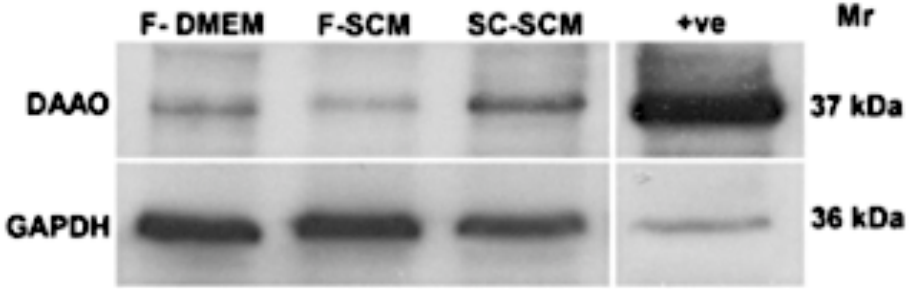
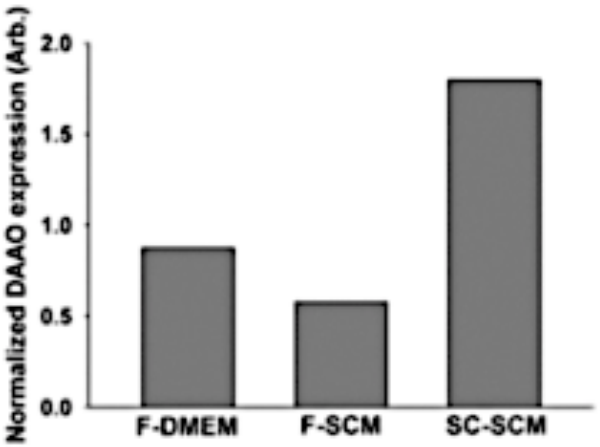
Schwann cells

Differential expression of *D-amino acid oxidase* between Schwann and fibroblasts identified









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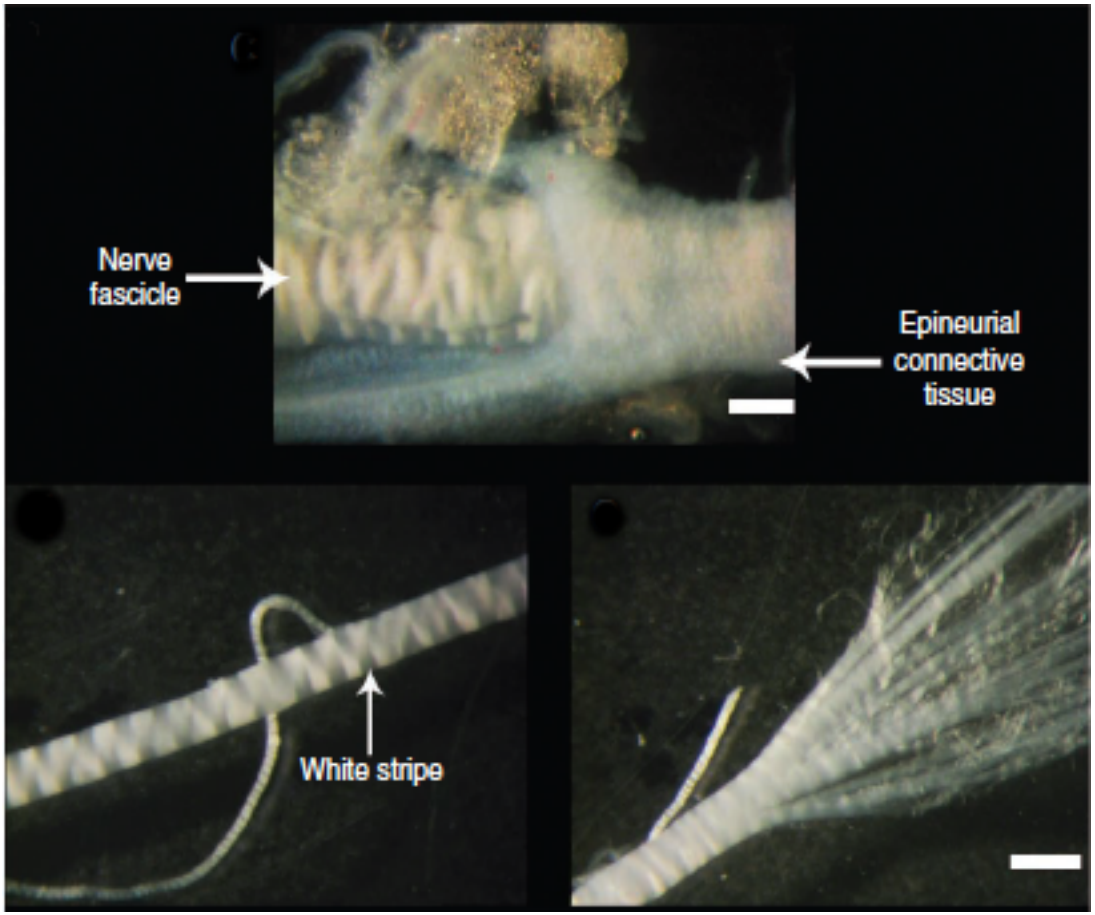
Schwann cells

Isolation from adult nerve



- Adult sciatic nerve isolated
- Epineurium removed
- Fascicles placed in collagenase
- Digest placed in DMEM D-valine + mitogens
- Change medium at day 7 – then every 3 days
- Check purity at days 19-21

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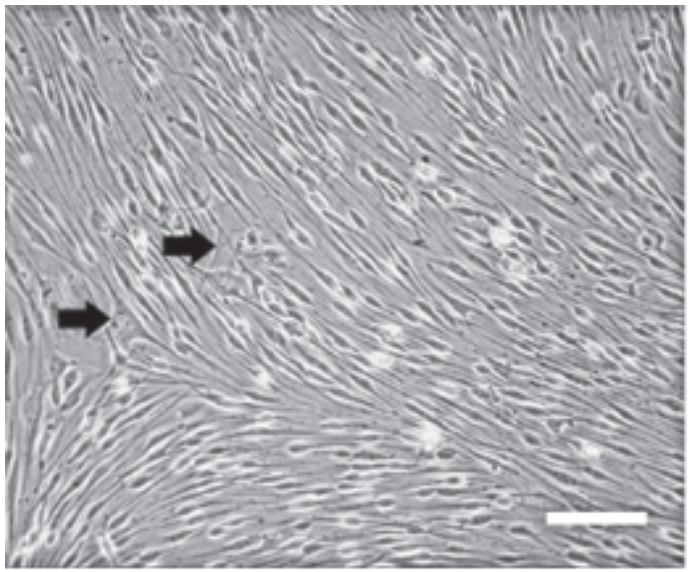
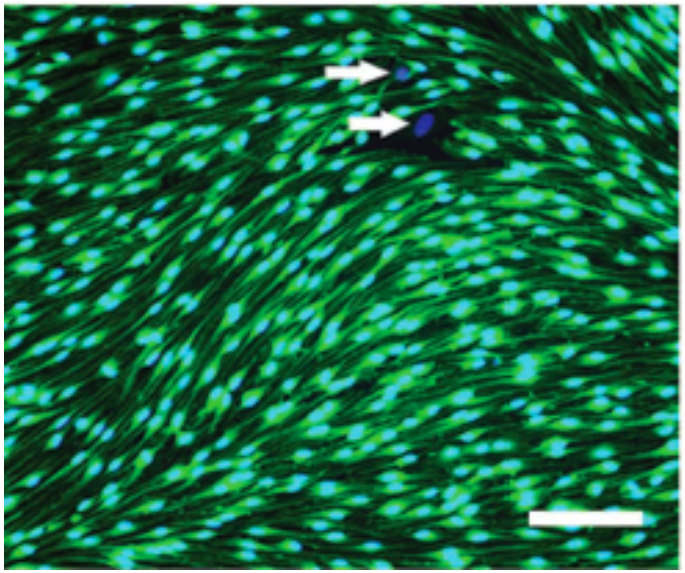
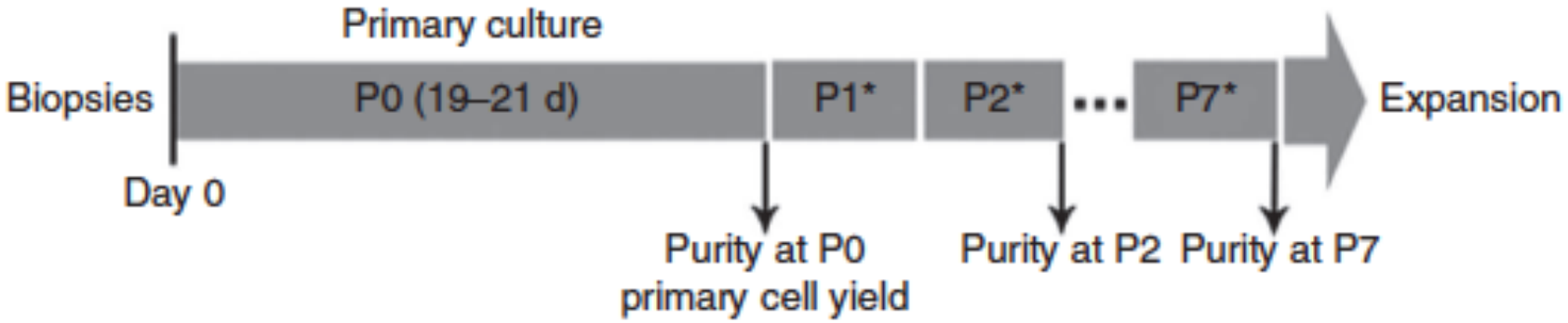
Schwann cells







97% **99-100%** **100%**

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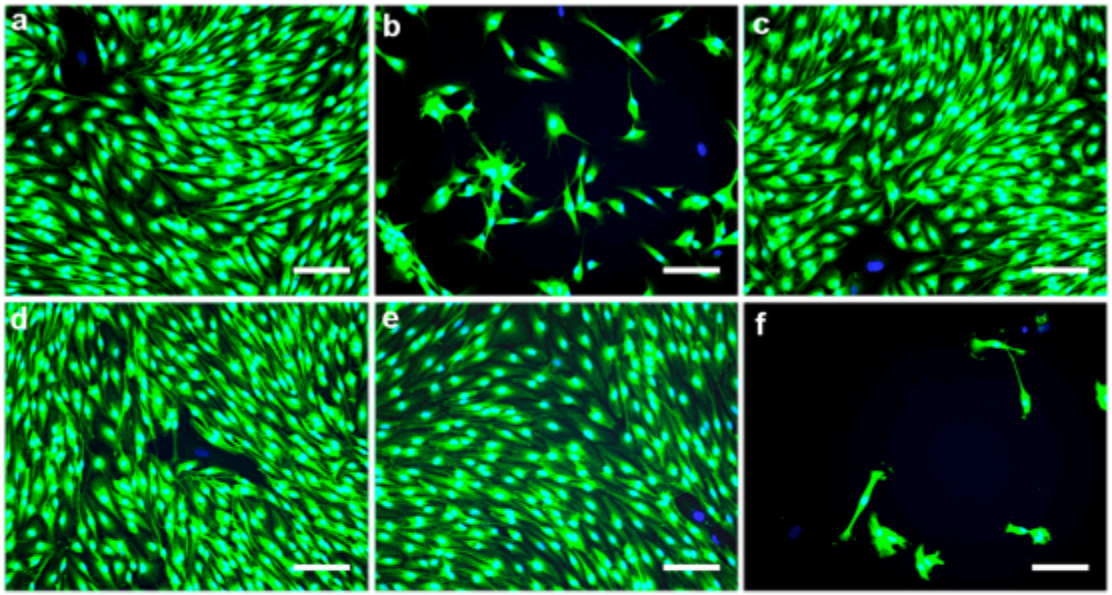
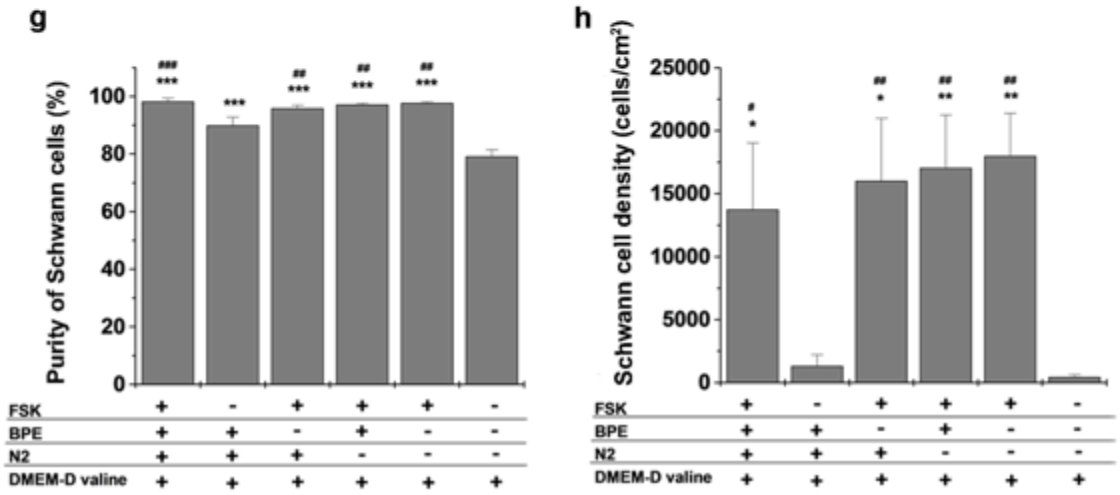
Schwann cells





- Mitogen combinations + DMEM D-valine

1. Forskolin
2. Bovine pituitary extract
3. N2 supplement

- **DMEM D-valine + forskolin**

- Kaewkhaw R, Scutt AM & Haycock JW (2012) A rapid method for the selective isolation of Schwann cells from adult nerve. ***Nature Protocols*** 7, 1996–2004.

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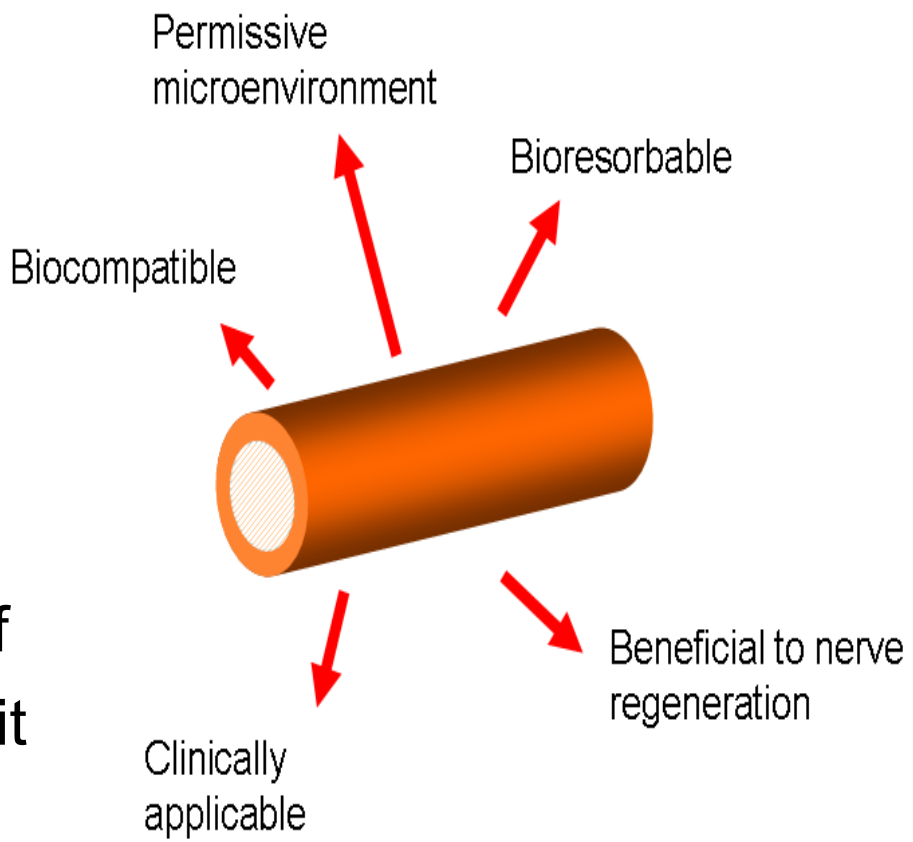

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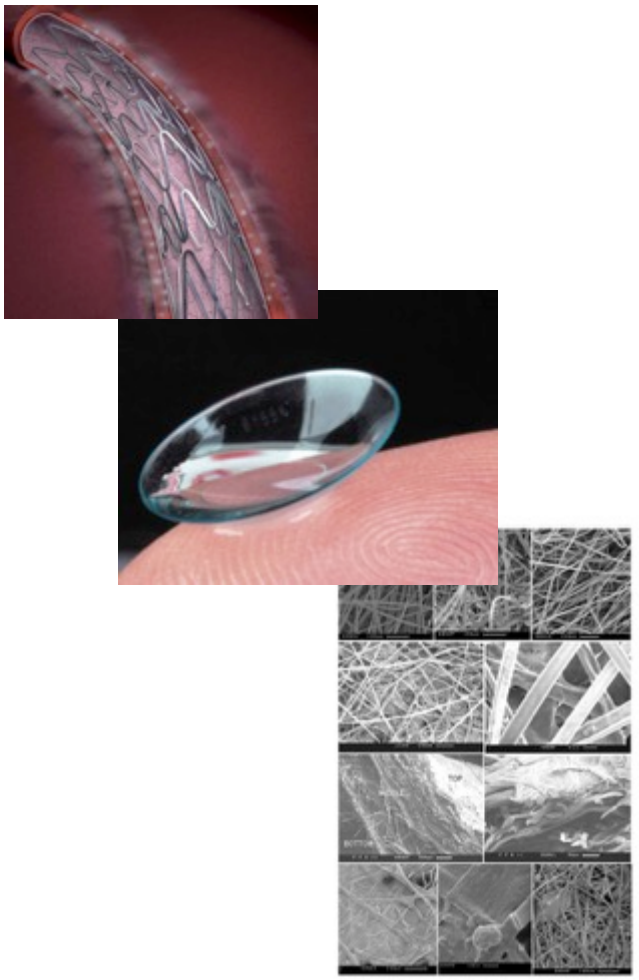
Bioactive surfaces

‘Dip & dry’ anti-inflammatory biomaterials
Anti-microbial biomaterials

- To improve clinical outcome of coronary stenting
- To reduce inflammation / infection problems of contact lenses
- To improve the success of tissue engineering approaches where inflammation / infection is a problem
- A quick and easy method for surface treatment



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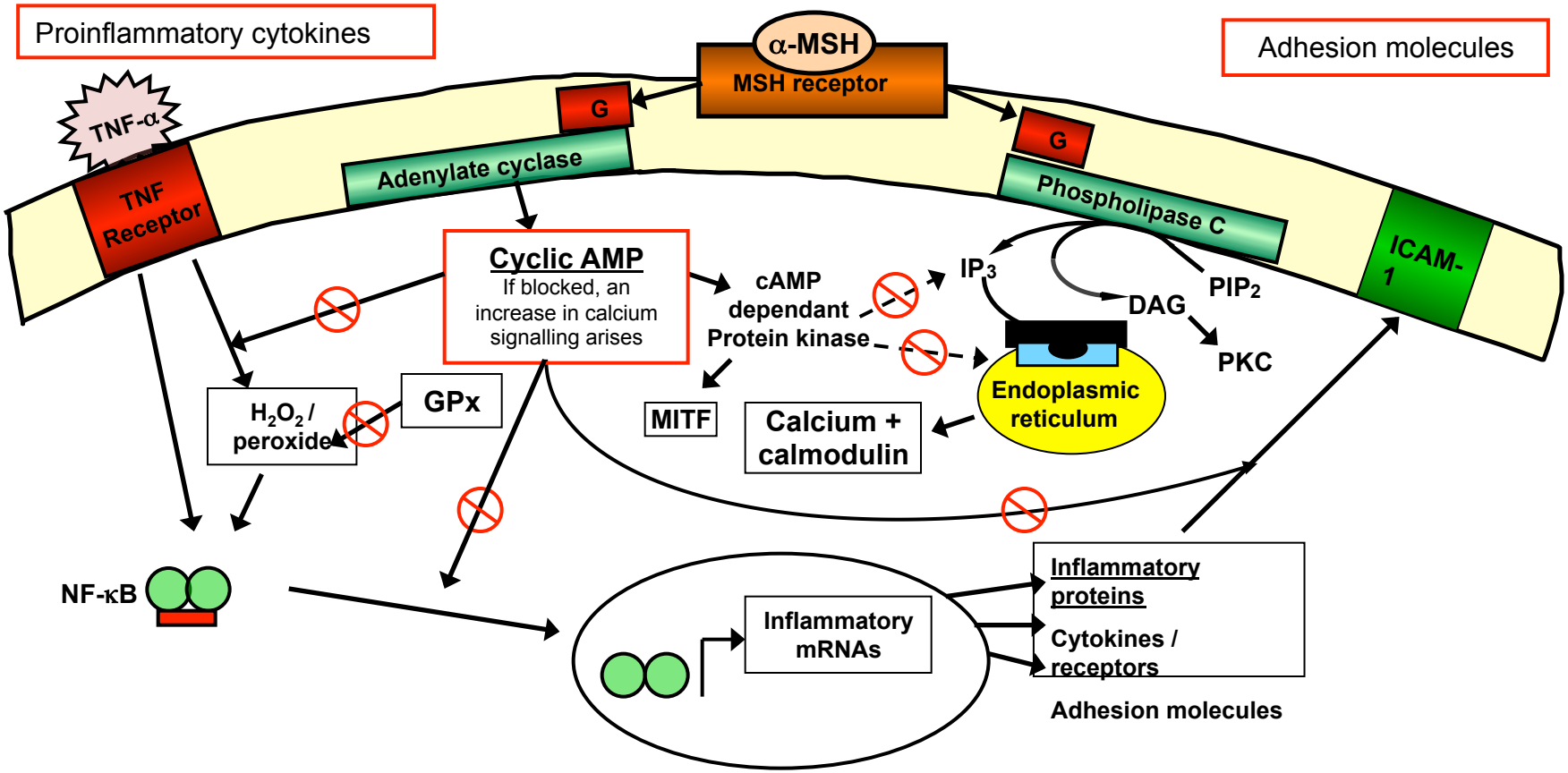
Anti-inflammatory peptide – α-MSH



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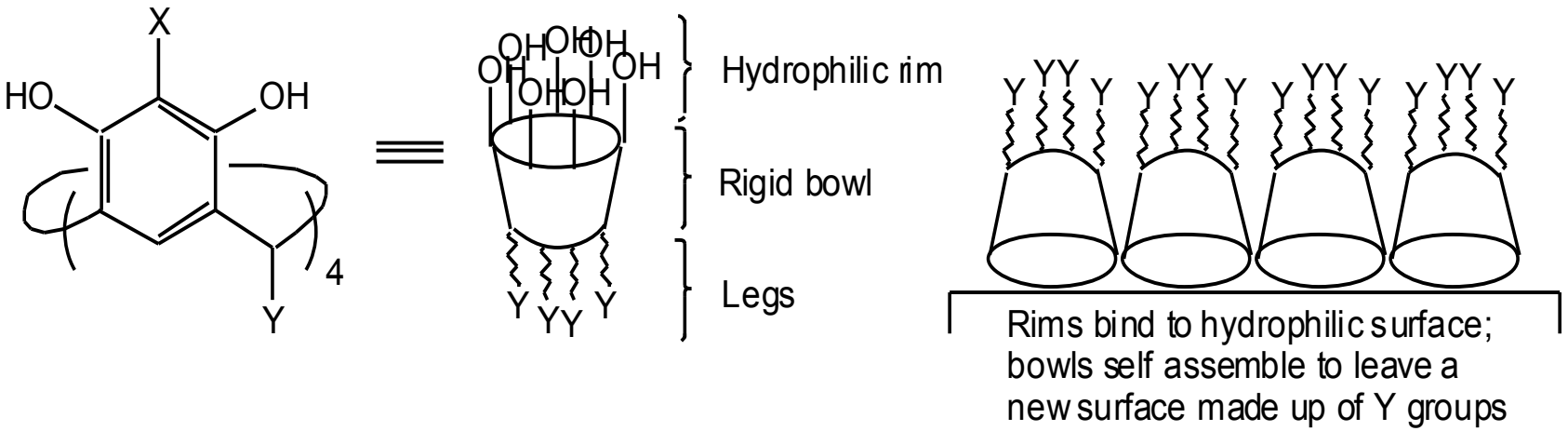
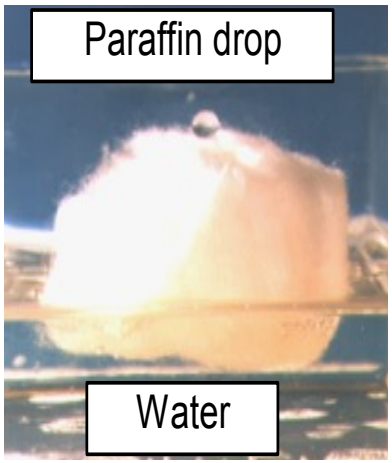
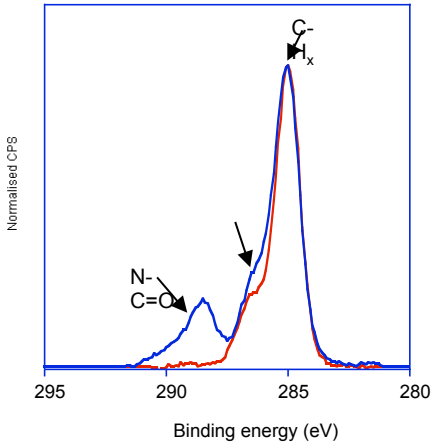
Rapid self-assembling adhesive molecules

Resorcinarenes

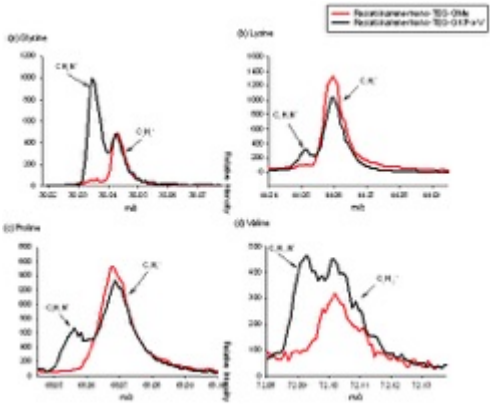




Synthesis of α-MSH anti-inflammatory peptide – resorcinarene conjugates

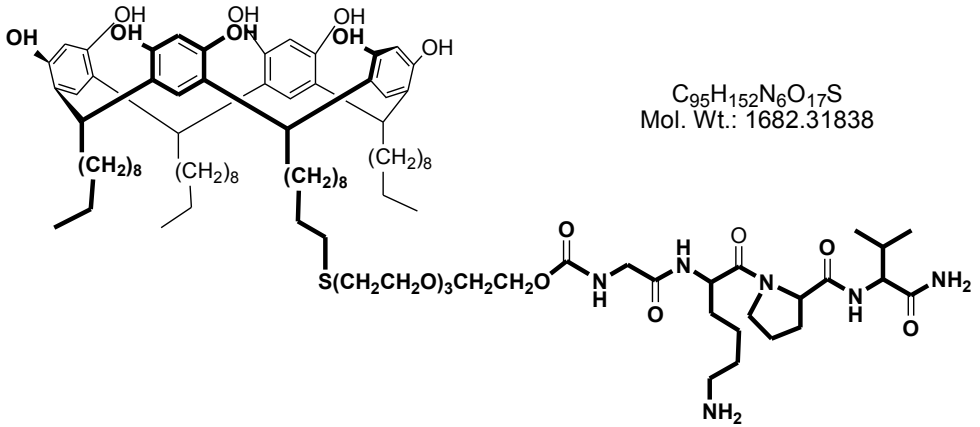


X-Ray Photoelectron Spectroscopy



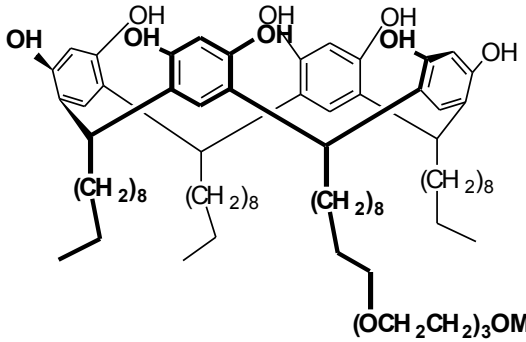
Surface Ion Mass Spectrometry

Calixarene-monoPEG-GKP(D)V



$C_{95}H_{152}N_6O_{17}S$
Mol. Wt.: 1682.31838

Calixarene-monoPEG-OMe



$C_{75}H_{118}O_{12}$
Mol. Wt.: 1211.73222

Fairfull-Smith K, Redon PMJ, Haycock JW, Williams, NH (2007).
Tetrahedron Letters, 48: 1317-1319.

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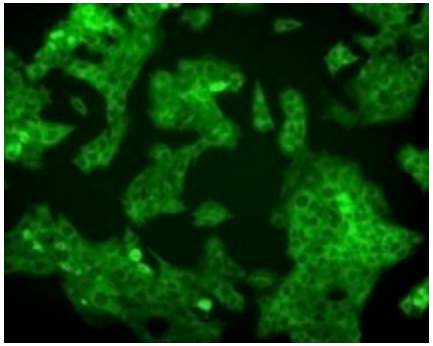
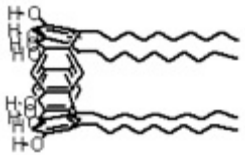
Calixarene-peptide surfaces

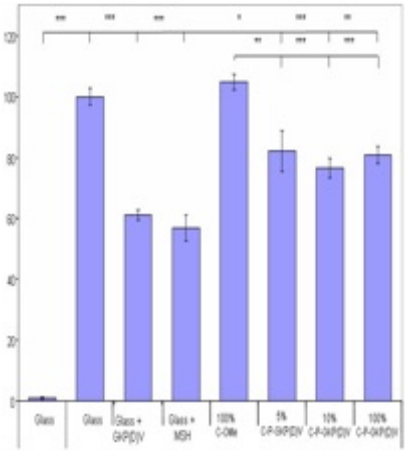
NF-kB labelling in nerve Schwann cells

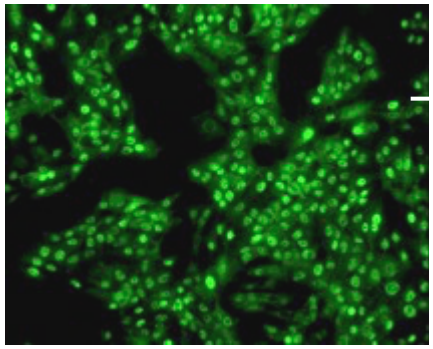


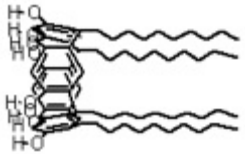


Calixarene-monoPEG-OMe

No TNF-α

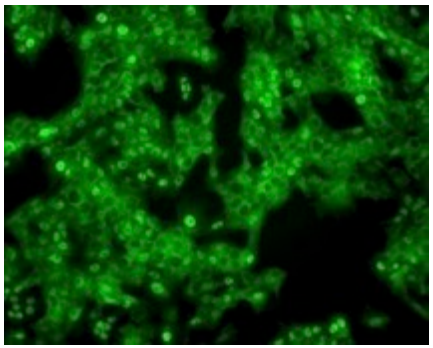


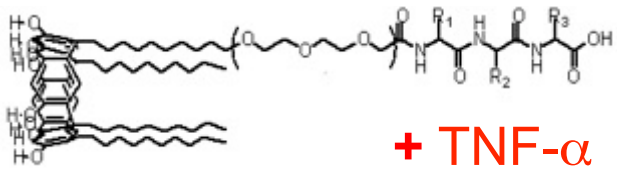




Calixarene-monoPEG-OMe

+ TNF-α





+ TNF-α

Calixarene-mono PEG-**GKP(D)V**

Charnley M, McArthur SL, Williams NH, Haycock JW. (2009). **Advanced Materials,** 21(28): 2909-2915.

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Recent + key publications

- Zilic L, Wilshaw SP, Haycock JW (2016) Decellularisation and histological characterisation of porcine peripheral nerves. ***Biotechnology & Bioengineering***. (In press).
- Stevenson G, Rehman S, Draper E, Hernández-Nava, E. Hunt J and Haycock JW (2016) Developing 3D human in vitro methods for evaluating novel porous titanium surfaces in orthopaedic applications***. Biotechnology & Bioengineering***. (In press).
- Hopper, AP, Dugan, JM, Gill, AA, Regan, EM, Haycock, JW, Kelly, S, May, PW, Claeyssens, F (2016) Photochemically modified diamond-like carbon surfaces for neural interfaces. ***Materials Science and Engineering C*** 58(5725); 1199-1206.
- Htwe, SS, Harrington, H, Knox, A, Rose, F, Aylott, J, Haycock, JW, Ghaemmaghami, AM (2015) Investigating NF-kB signalling in lung fibroblasts in 2D and 3D culture systems. ***Respiratory Research*** (In press).
- Zilic L, Garner PE, Yu T, Roman S, Haycock JW, Wilshaw SP. (2015) An anatomical study of porcine peripheral and its potential in nerve tissue engineering. ***Journal of Anatomy*** 227(3); 302-314.
- Scherer, KM., Bisby, RH, Botchway, SW., Hadfield, JA., Haycock, JW., Parker, AW (2015) Three-dimensional imaging and uptake of the anticancer drug combretastatin in cell spheroids and photoisomerization in gels with multiphoton excitation. ***Journal of Biomedical Optics*** doi:10.1117/1.JBO.20.7.078003.
- Plenderleith RA, Pateman CJ, Rodenburg C, Haycock JW, Claeyssens F, Sammon C, Rimmer S (2015) Arginine-glycine-aspartic acid functional branched semi-interpenetrating hydrogels. ***Soft Matter*** 11(38); 7567-7578.
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- Hopper, A.P., Dugan, J.M, Gill, A.A., Fox, O.J.L., May, P.W., Haycock, J.W., Claeyssens, F. (2014) Amine functionalized nanodiamond promotes cellular adhesion, proliferation and neurite outgrowth. ***Biomedical Materials***, 9(4); 045009.
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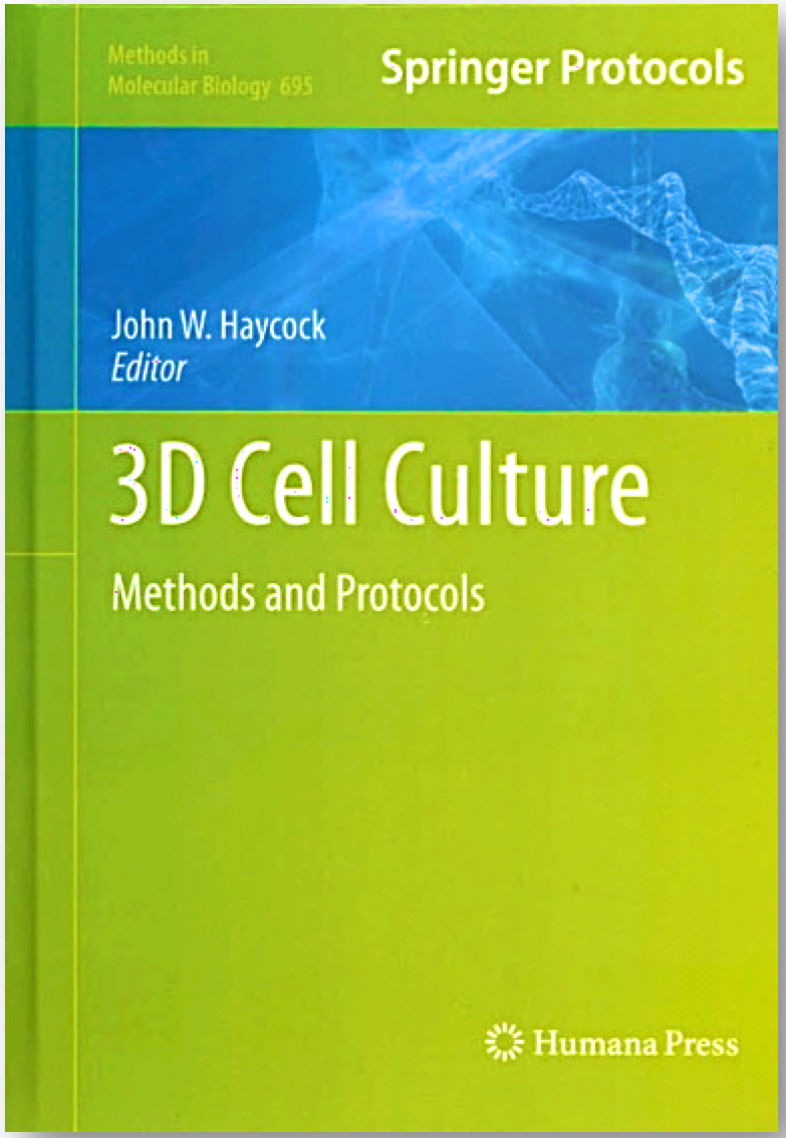
3D Cell Culture - books



- **3D Cell Culture -** Methods and Protocols
- John W. Haycock (Editor)
- 20 Chapters / 60 authors

- 1st Edition (2011) - 343 pages
- 104 illustrations
- ISBN: 978-1-60761-983-3
- A Humana Press product

Selected chapters

- A Model Epithelial - Neuroectodermal - Mesenchymal Interaction System
- 3D Sample Preparation for Orthopedic Tissue Engineering Bioreactors
- Stem Cell and Neuron Co-Cultures for the Study of Nerve Regeneration
- Organotypic and 3D Reconstructed Cultures of the Human Bladder and Urinary Tract
- Alvetex®: Polystyrene Scaffold Technology for Routine Three Dimensional Cell Culture

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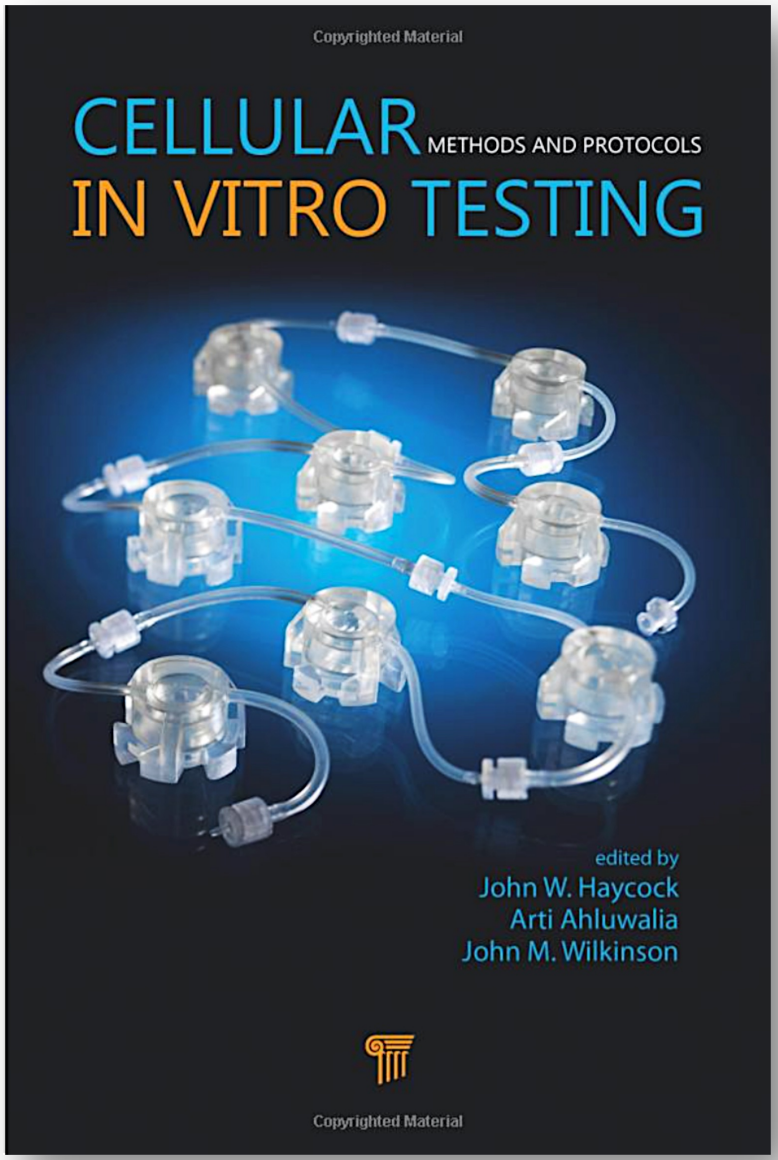
3D Cell Culture - books



- **Cellular In Vitro Testing**
- John W. Haycock
- Arti Ahluwalia
- J Malcolm Wilkinson

} Editors

- 10 Chapters / 40 authors
- 1st Edition (2014) - 155 pages
- 104 illustrations
- ISBN: 9789814364973
- Pan Stanford Press

Selected chapters

- Methods for Conducting Connected Culture Experiments Using the Quasi-Vivo R Chambers
- The Use of in vitro 3D Cell Models of Human Airway Epithelia (MucilAirTM) in Inhalation Toxicity
- Generation of Patient-Specific Cardiac Patches by Human Cardiac Progenitor Cells and 3D Scaffolds
- Utilizing Nanosensor-Incorporated Scaffolds in the Development of a 3D Lung Model

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