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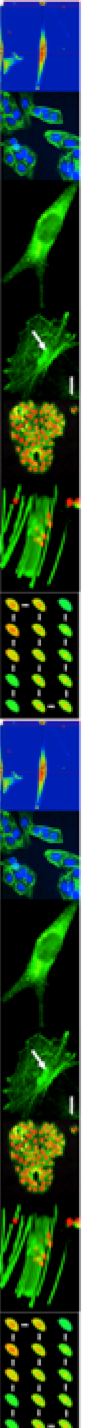

Bioengineered approaches for repairing nerve injury

John Haycock

Professor of Bioengineering

Head of Materials Science and Engineering
University of Sheffield, UK.

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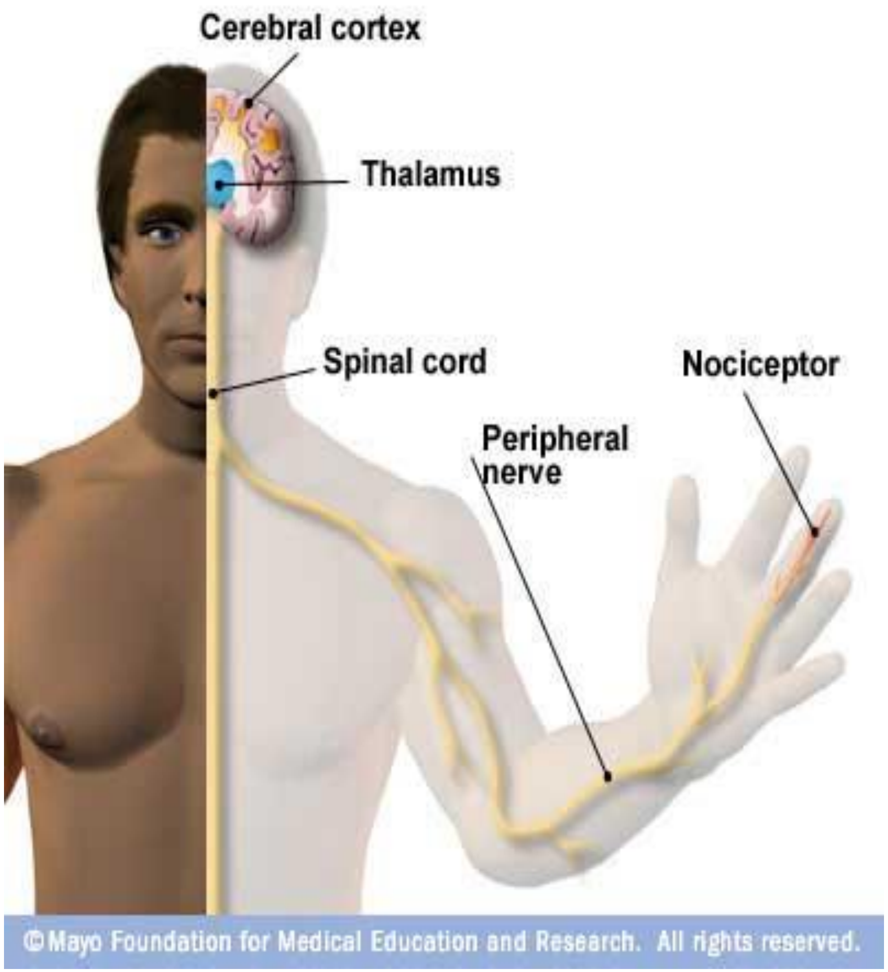
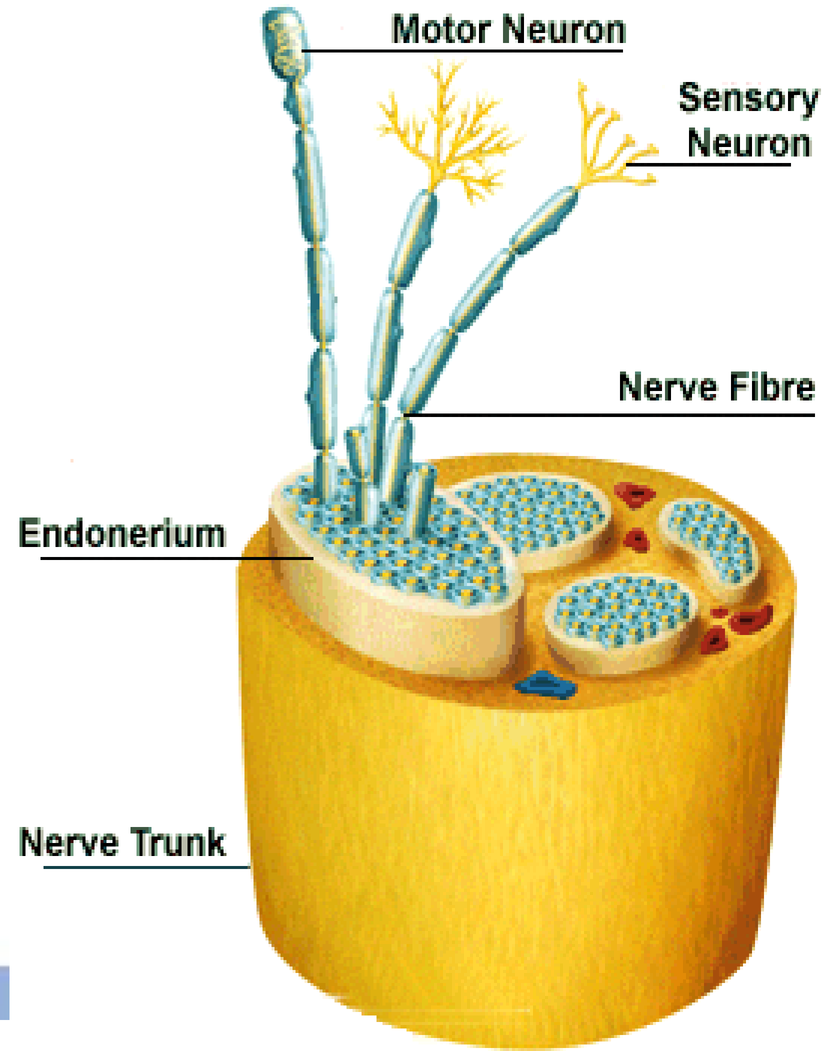
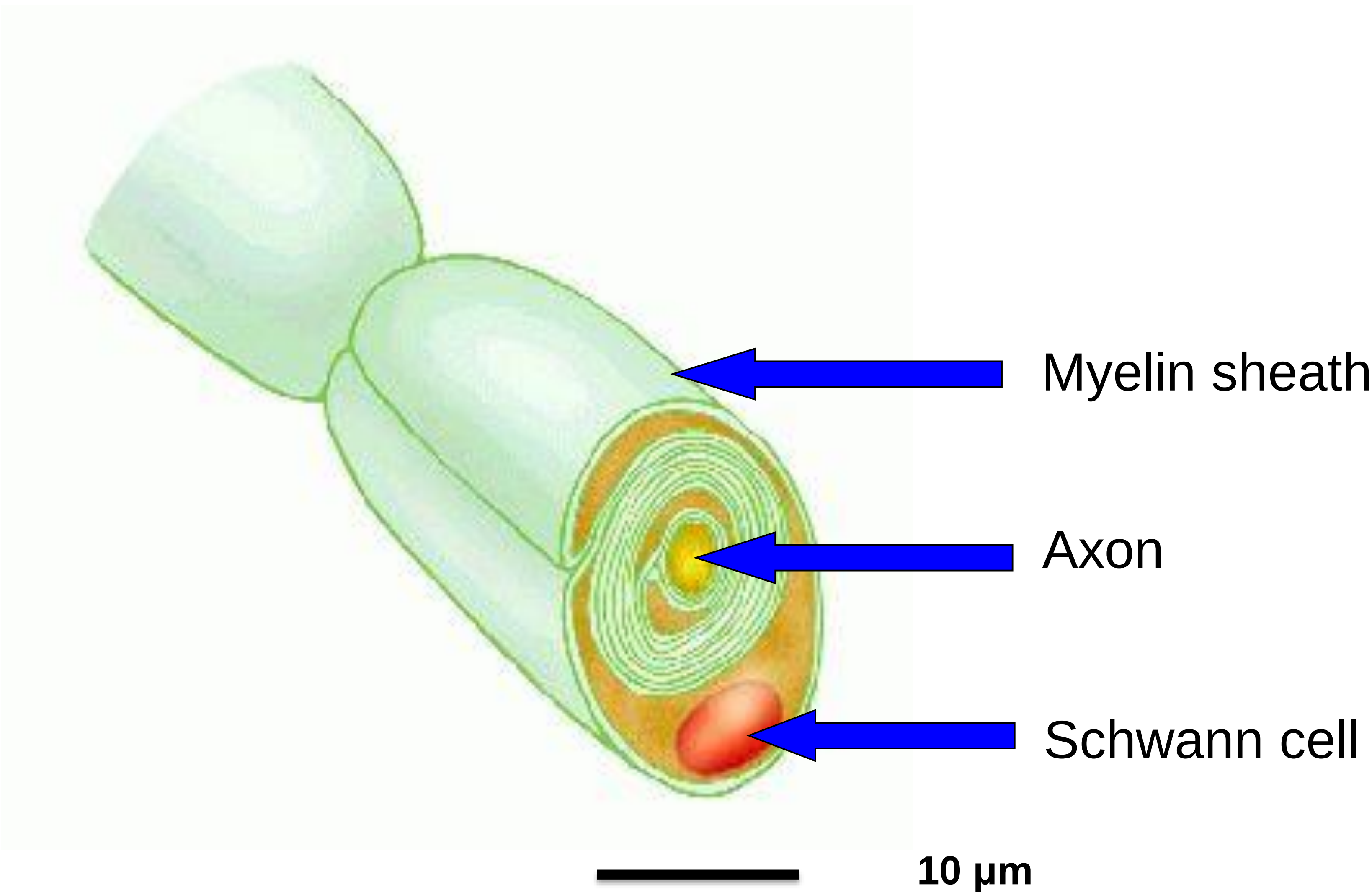
Peripheral nerve





Peripheral nerve axons

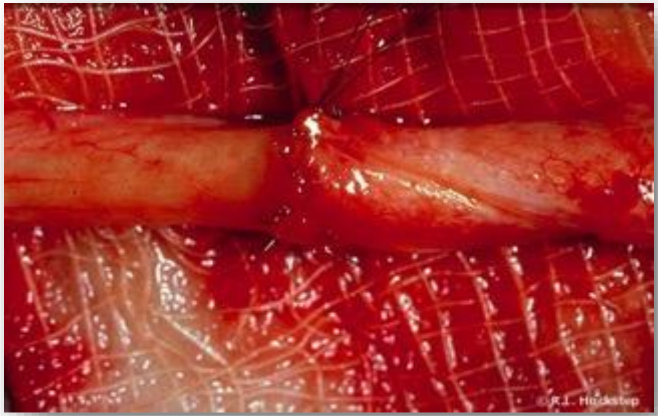


Three main clinical strategies to repair gap injuries

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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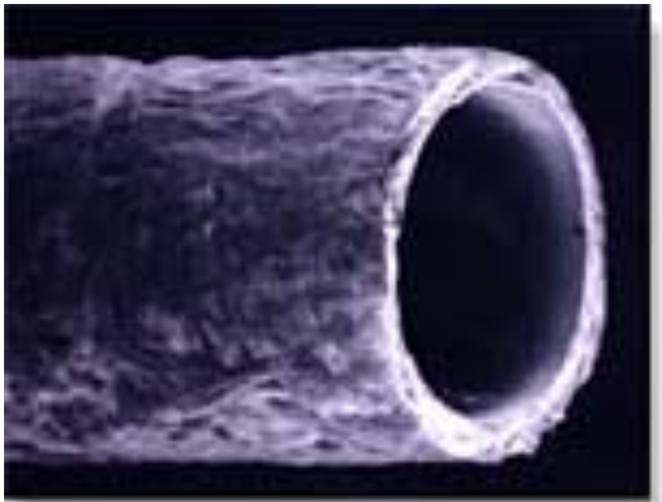
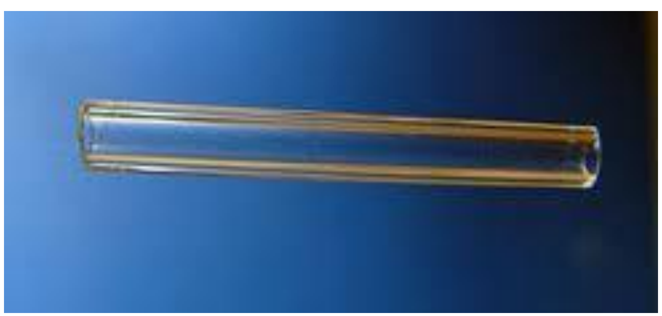
Nerve Guidance Channels



- 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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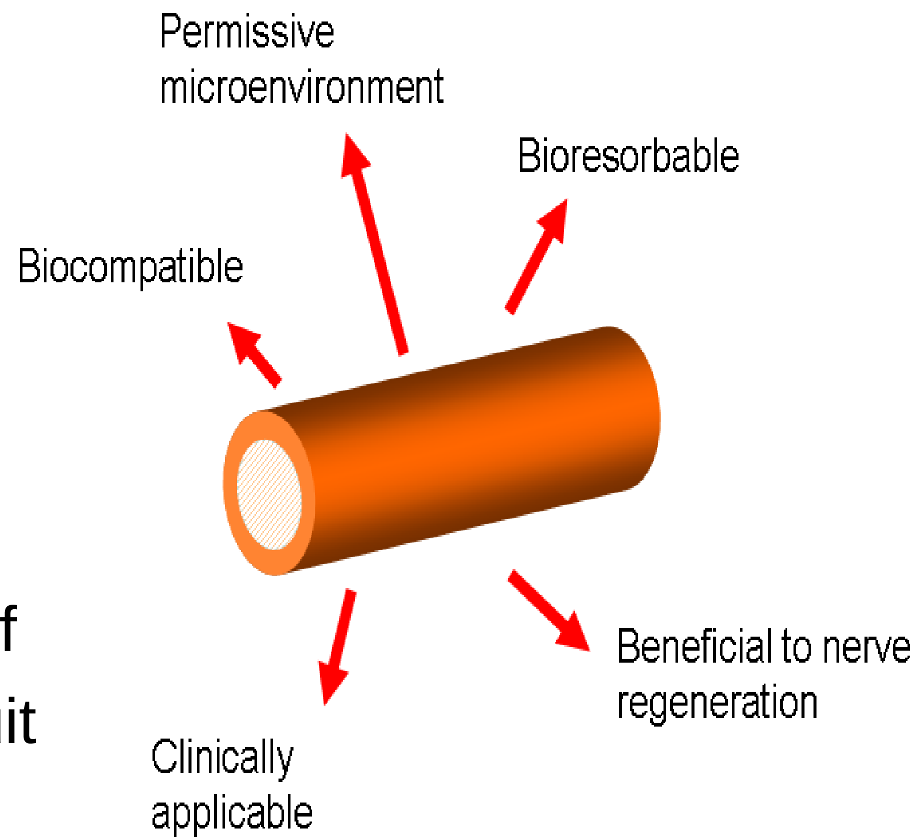
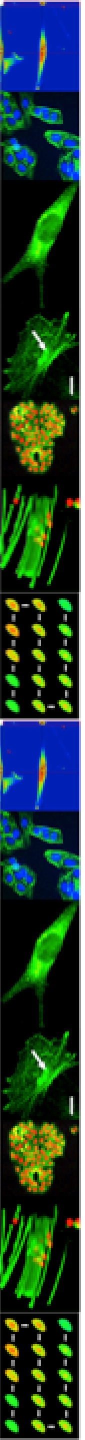
Present strategies for repairing peripheral nerve

- 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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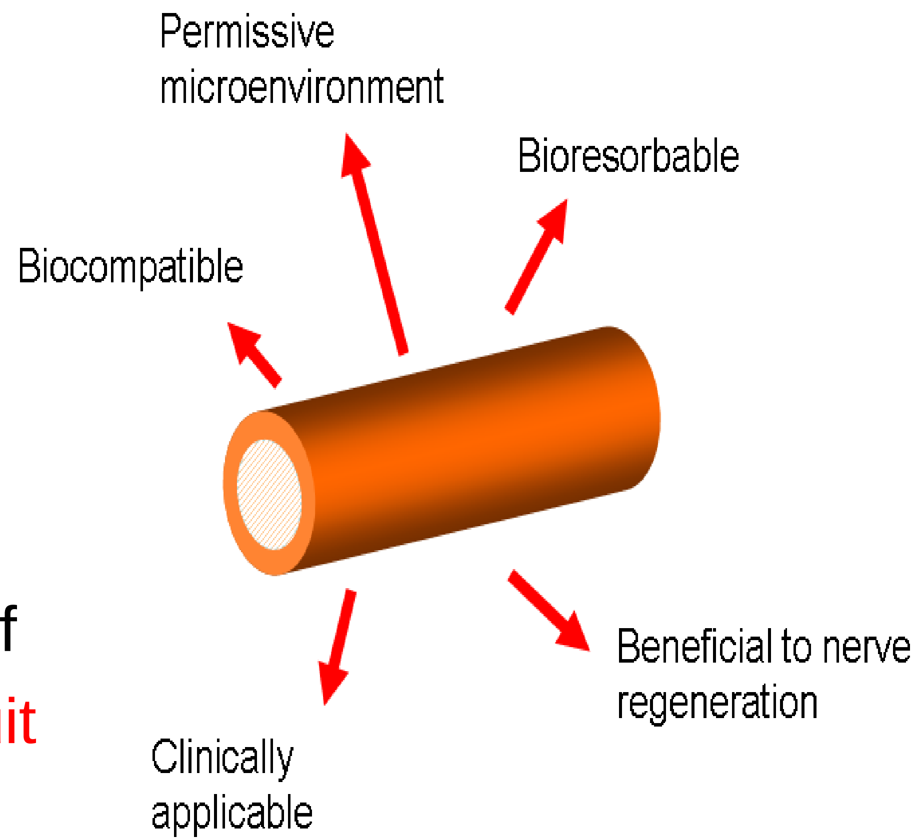
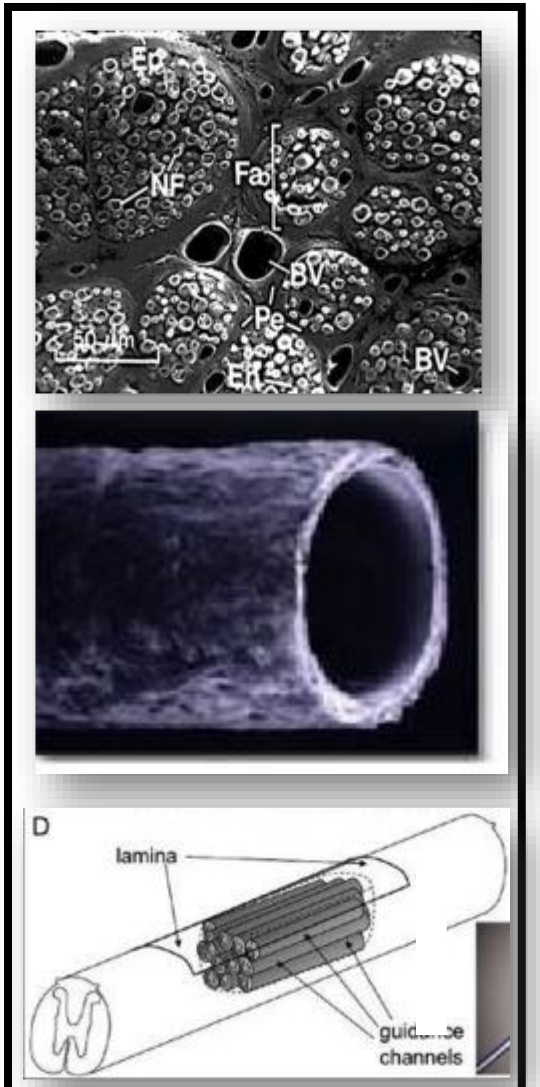
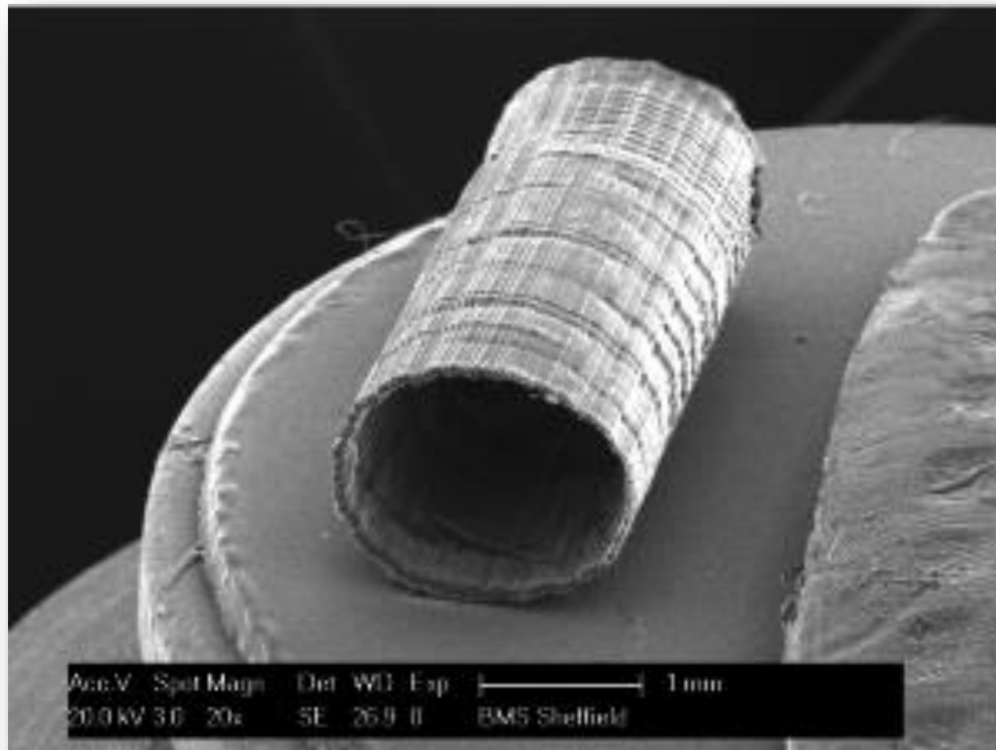


Making a scaffold precisely

Micro-stereolithography



1. Manufacture of NGCs - PEG, PLA, PCL, PGS
2. Incorporate internal structure within the tube to improve regeneration



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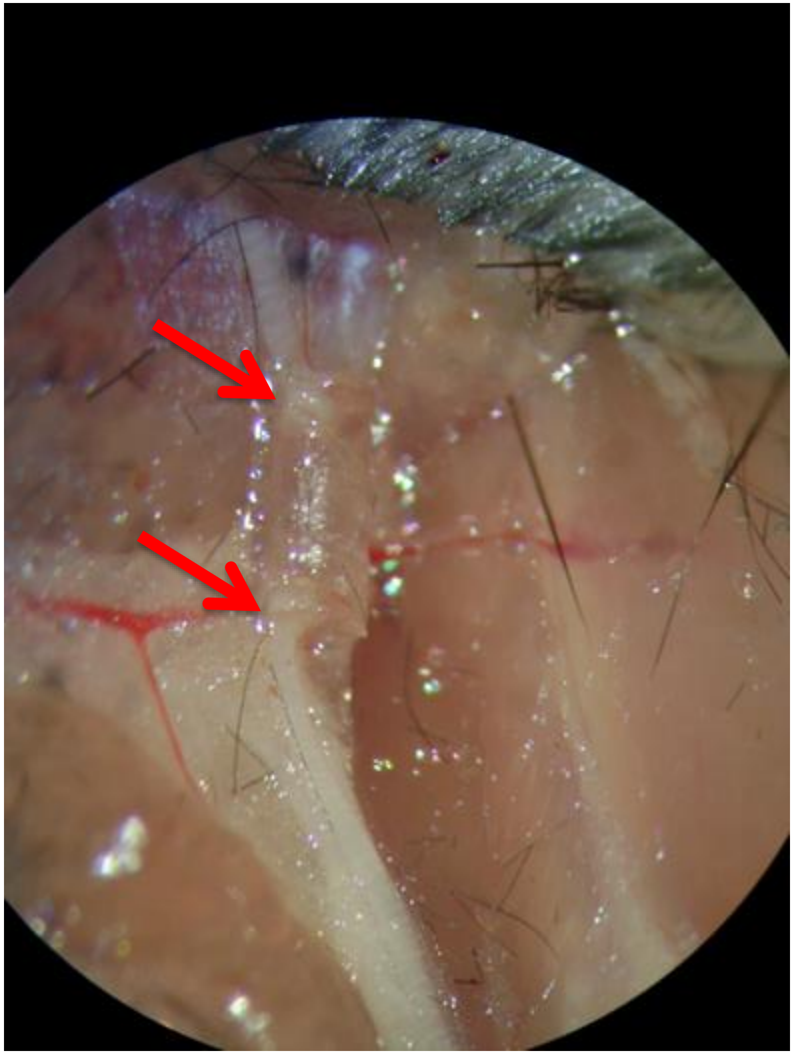
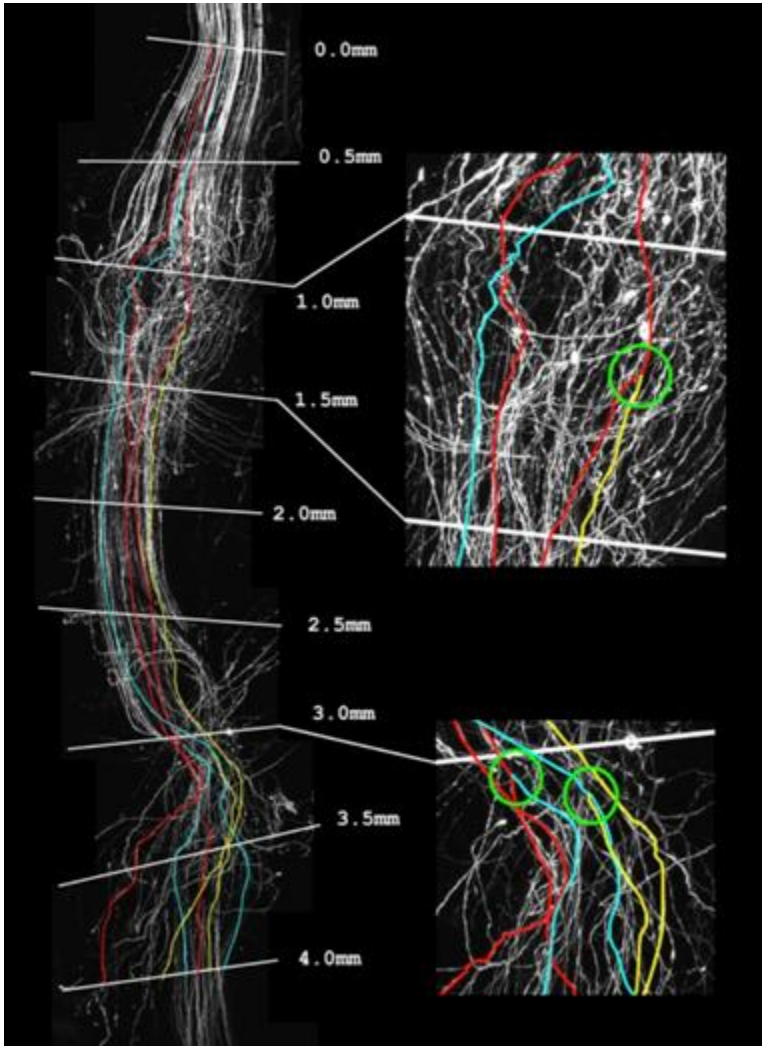
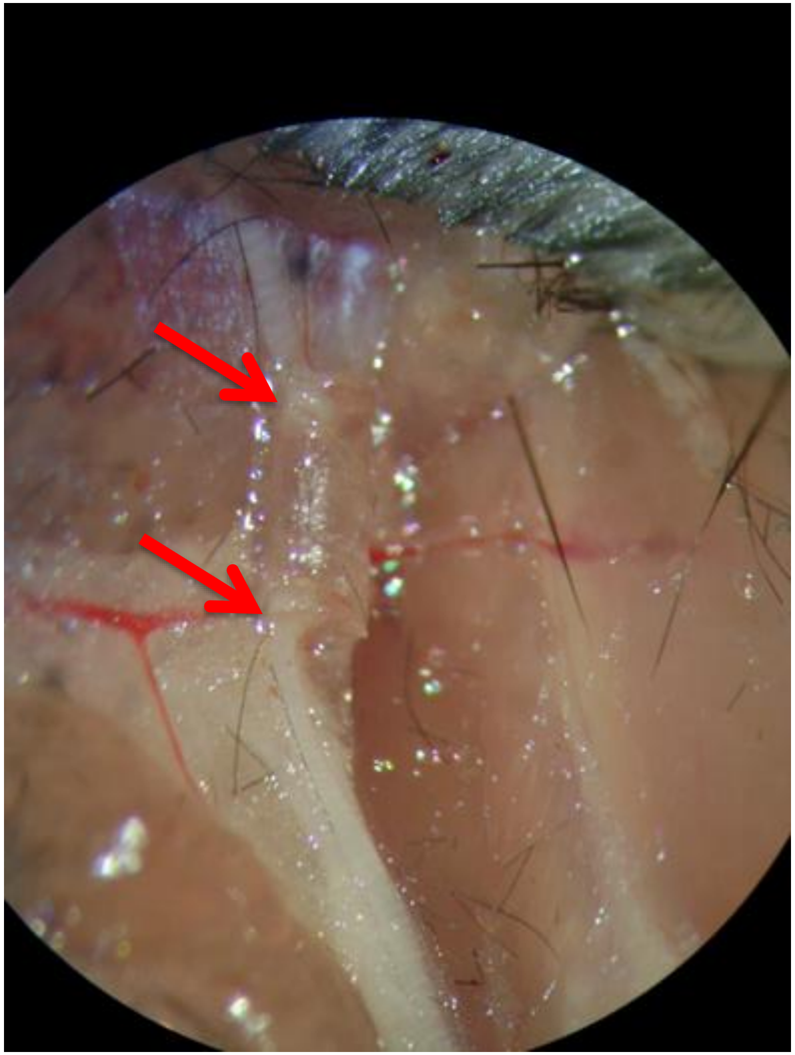
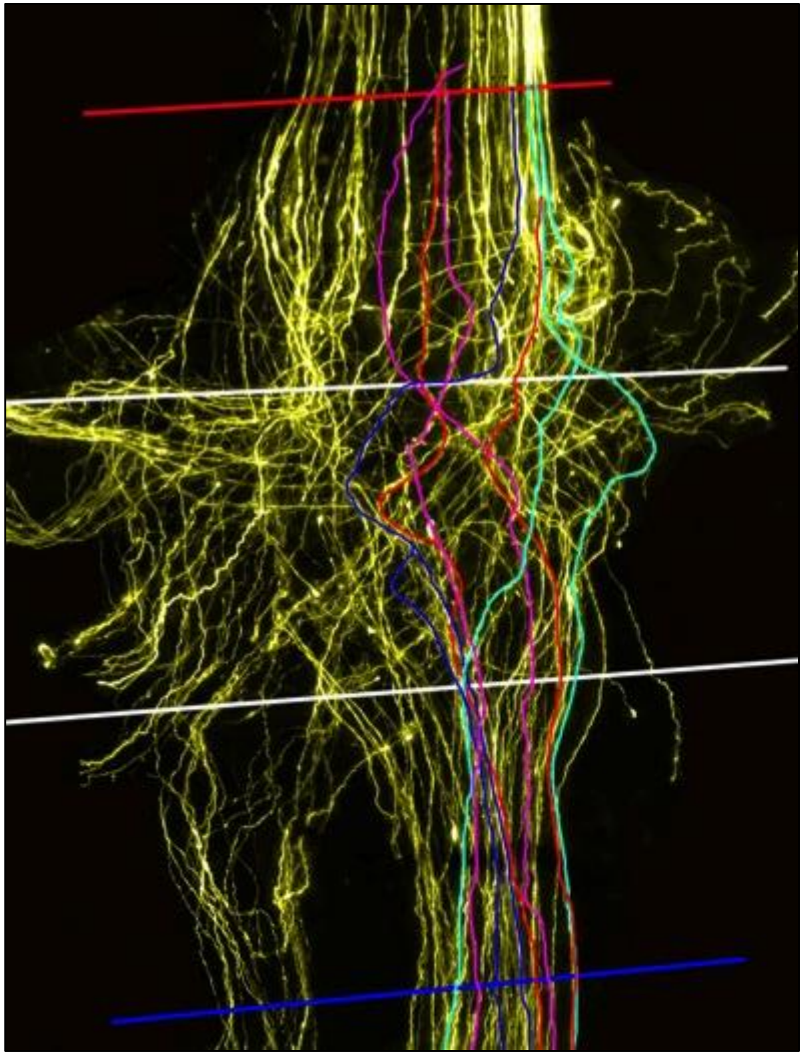
YFP mouse – 3mm common fibular nerve injury model



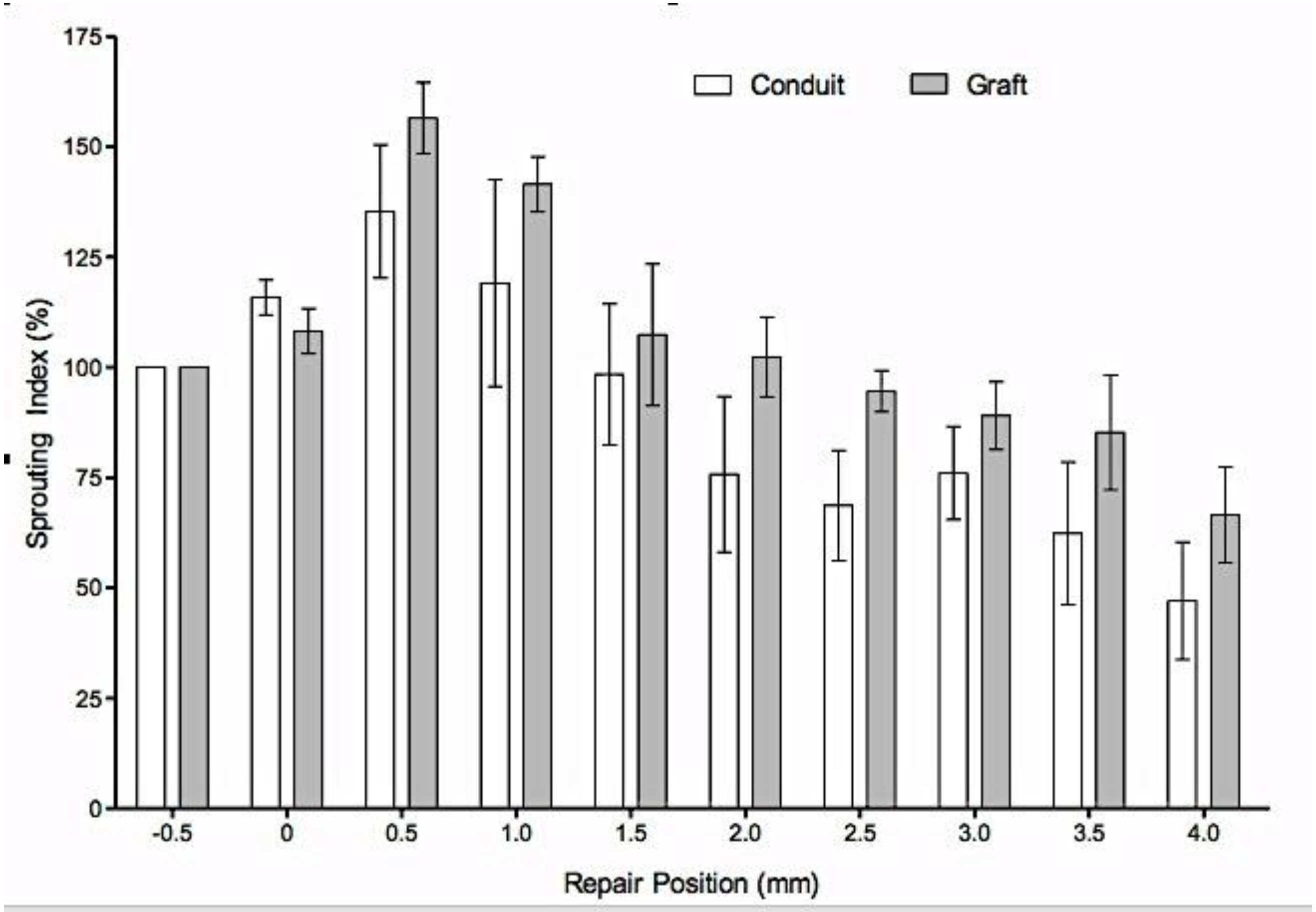


YFP mouse – 3mm common fibular nerve injury model





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. <u>Biomaterials</u> 49, 77–89.

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Present strategies for bioengineering peripheral nerve

- To increase regeneration distance
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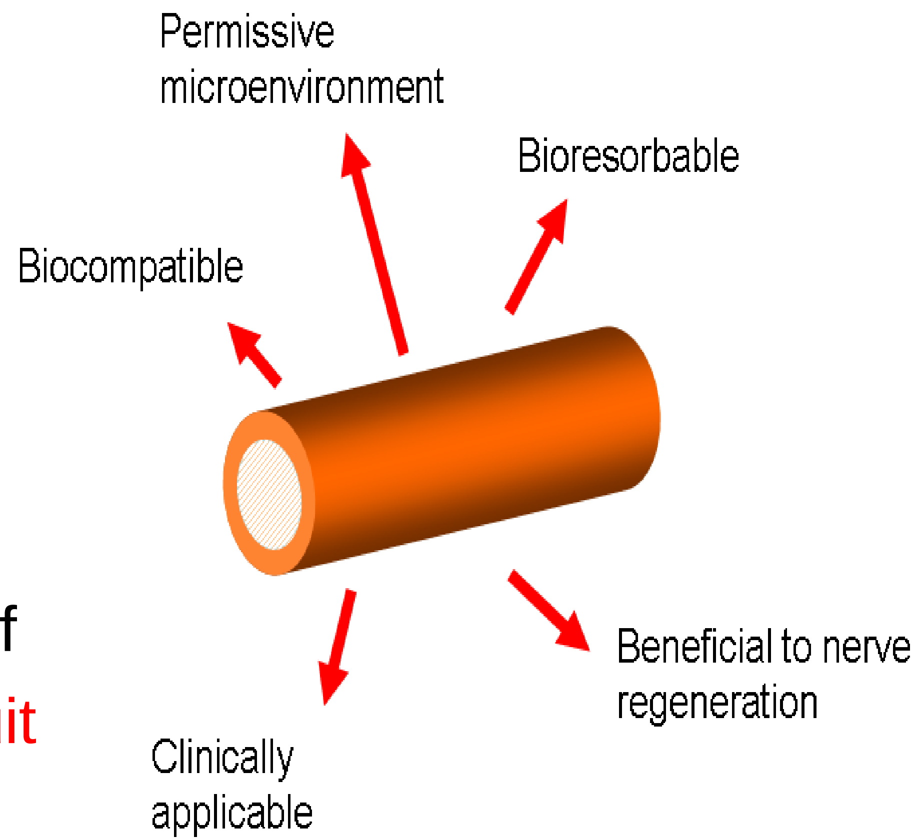

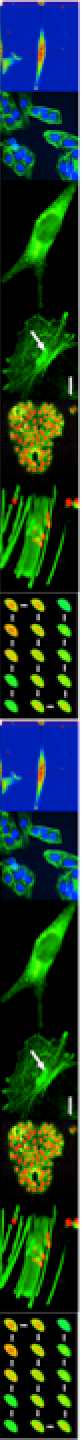

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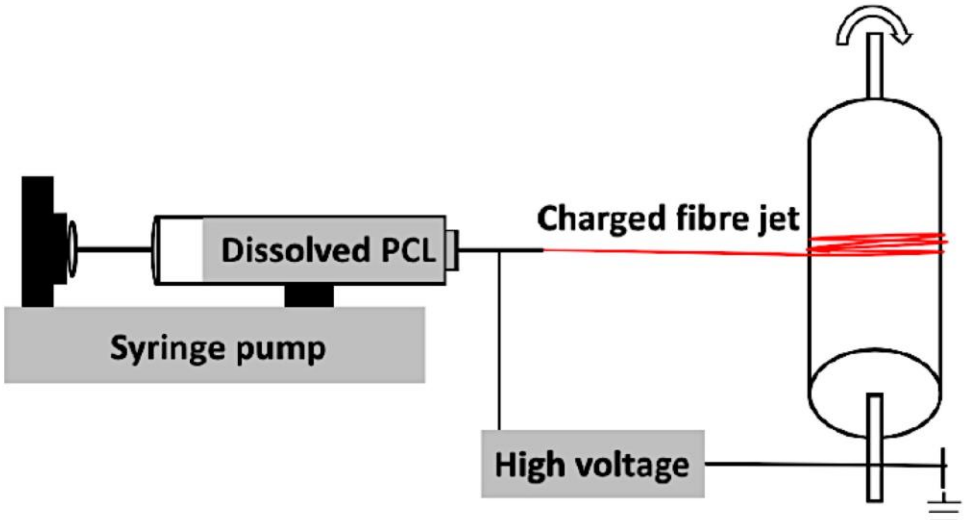
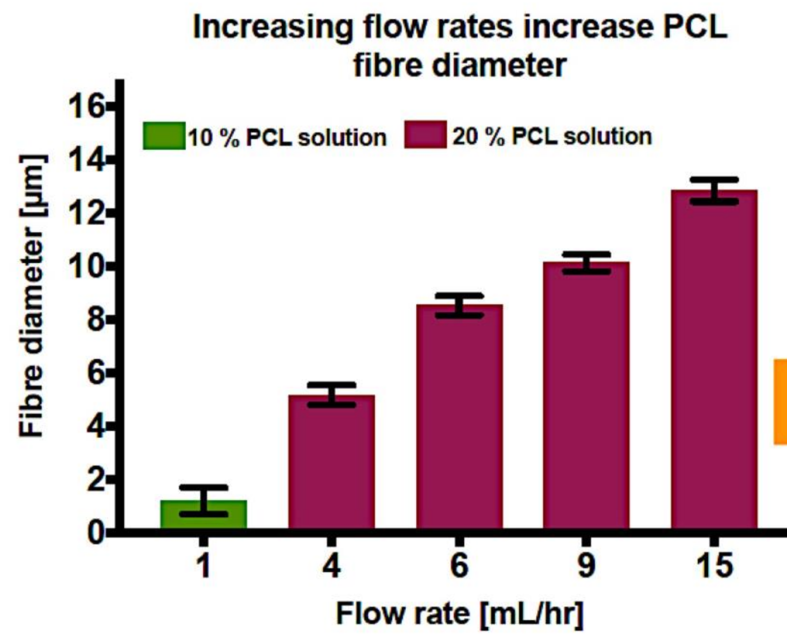
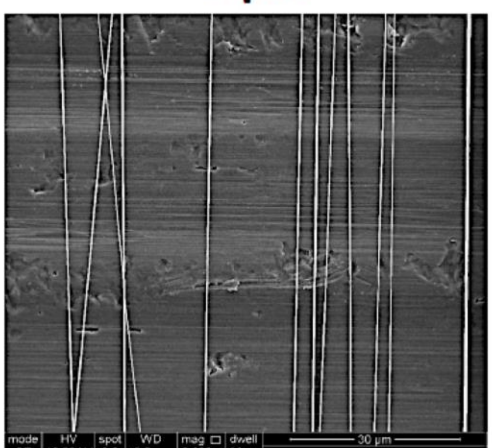
3D In vitro assessment model

Electrospinning of aligned PCL microfibres

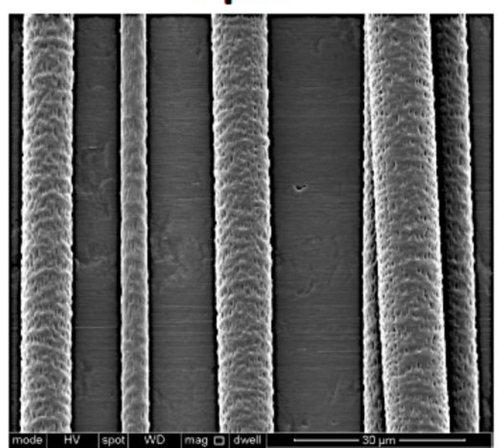




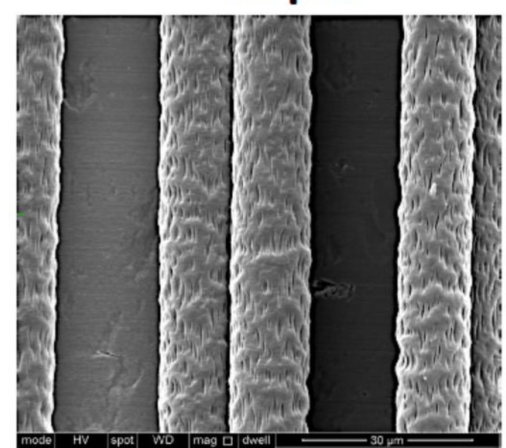
1 µm



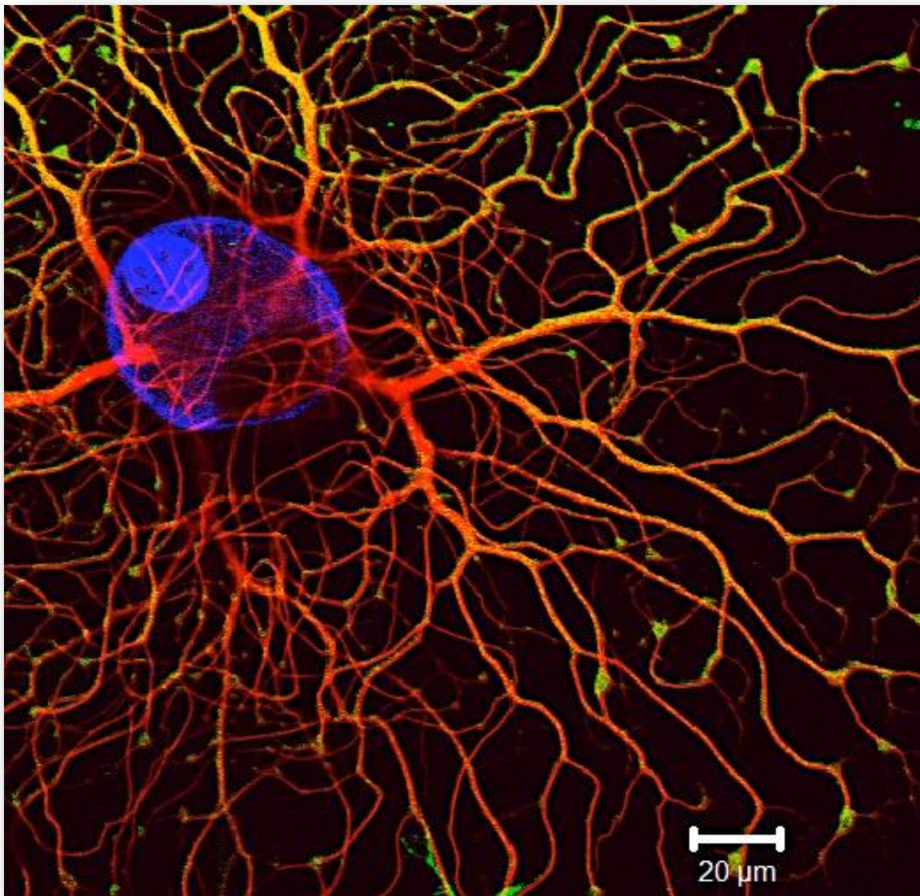
8 µm



13 µm



Dorsal Root Ganglion cultures

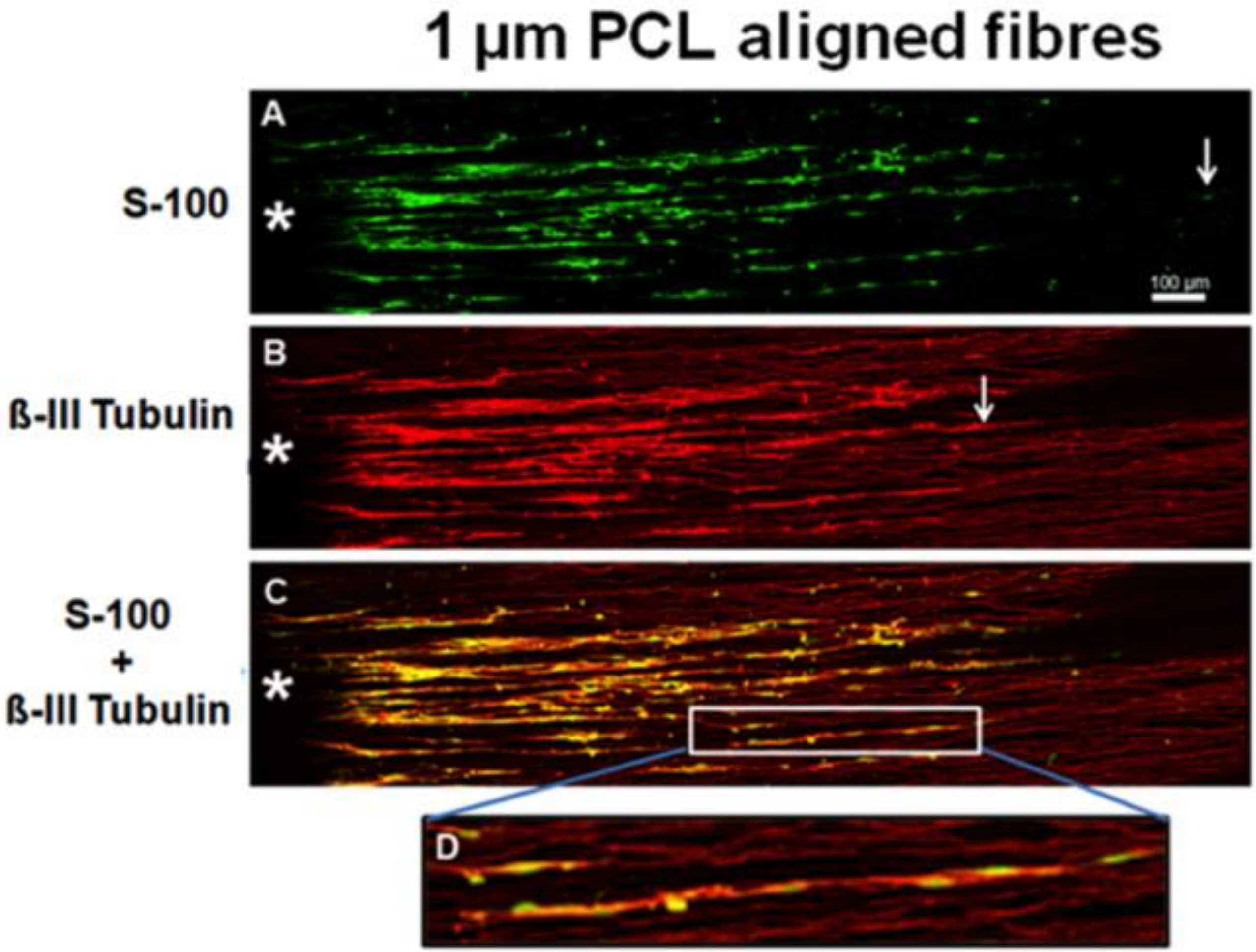


- On flat culture surfaces DRG neurites form a highly connected but disorganised network

- Nuclei
- β-tubulin-III
- S100β

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

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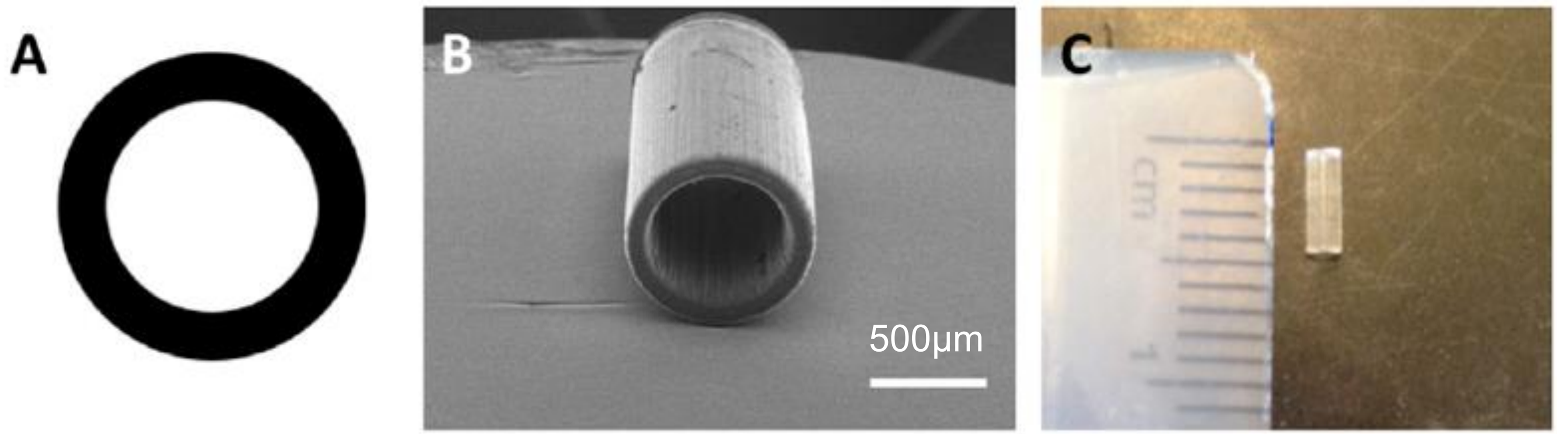
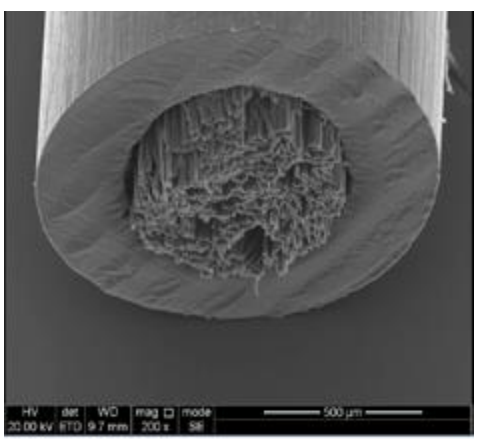
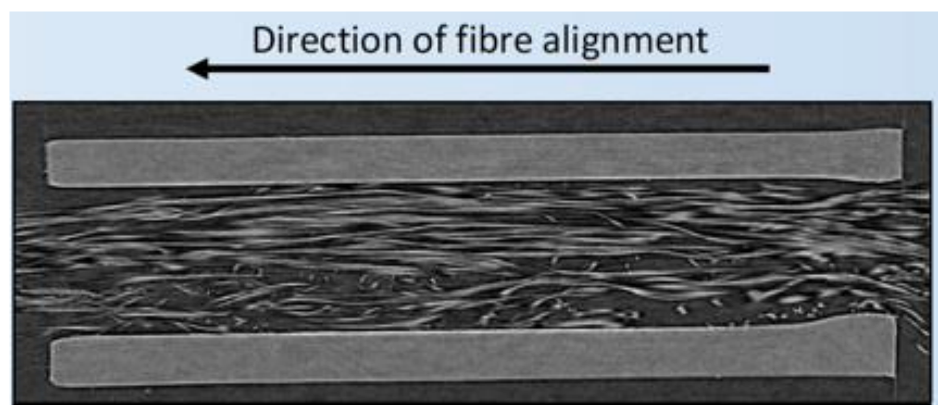
Photocurable poly(caprolactone) conduit + poly(caprolactone) fibres







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

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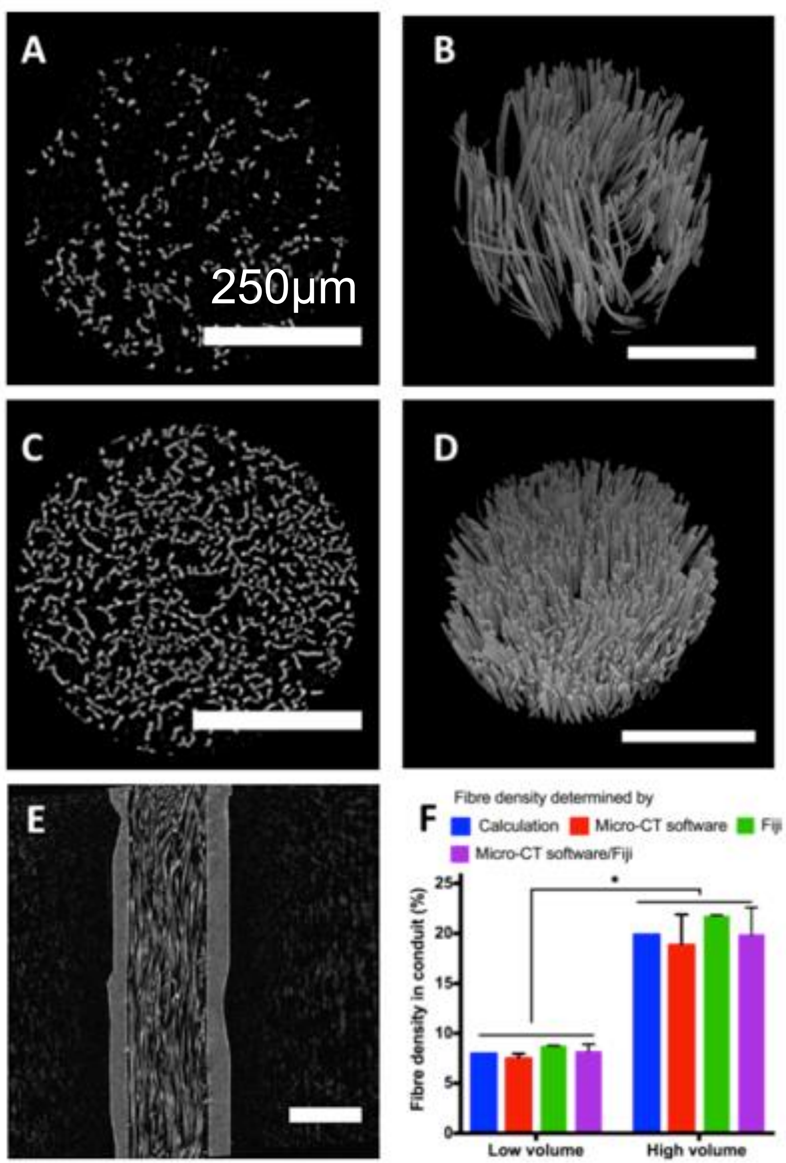
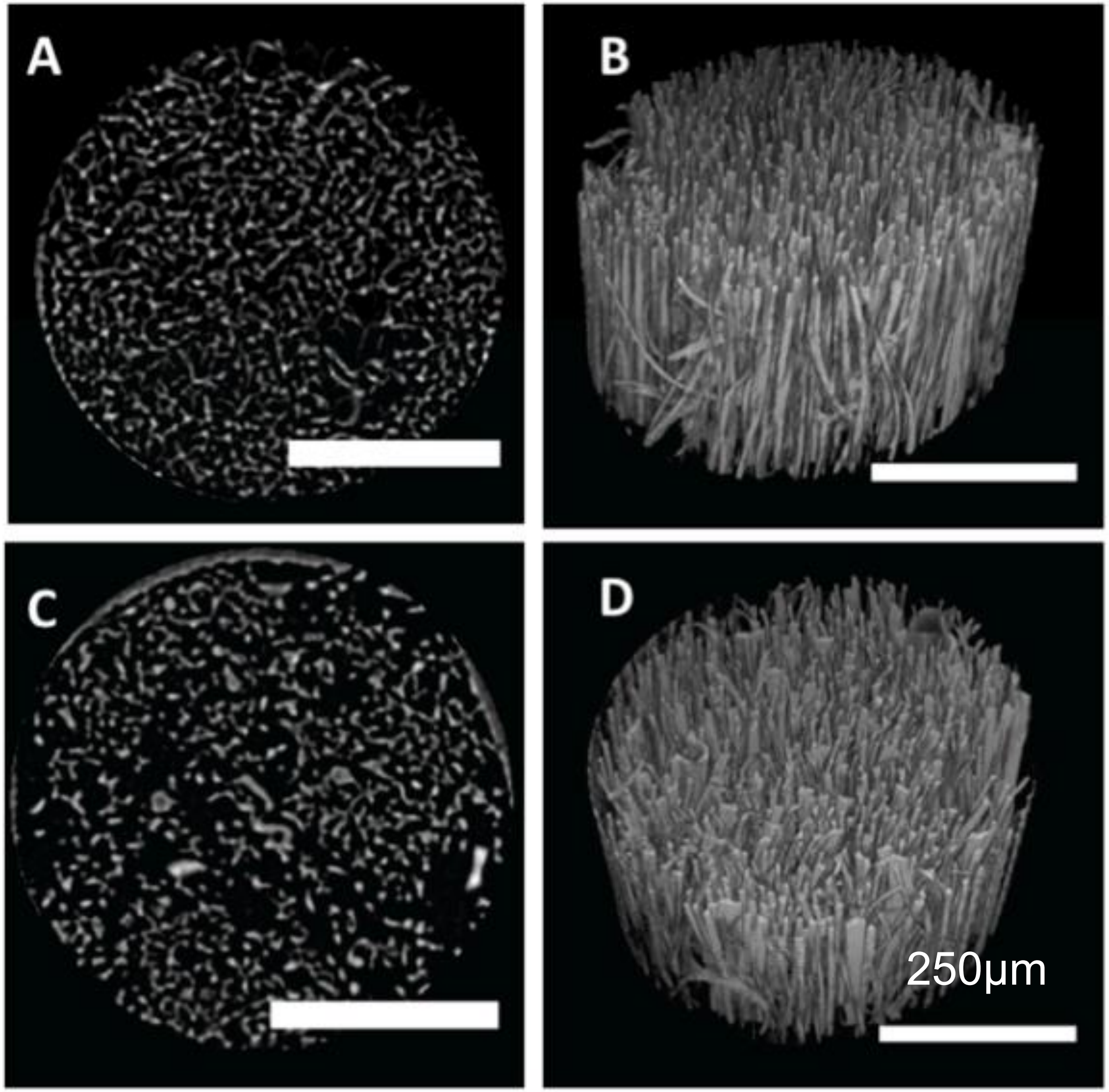
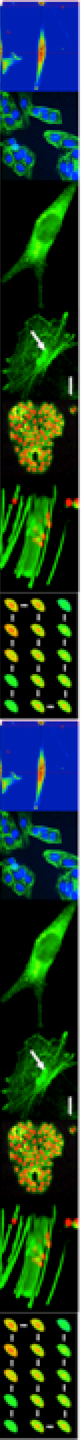


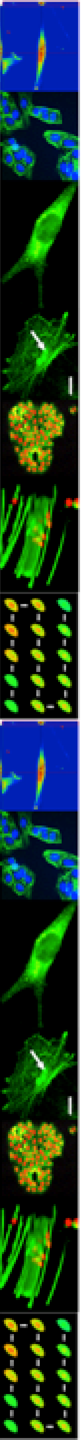
Micro CT imaging of NGCs

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



Micro CT imaging of NGCs





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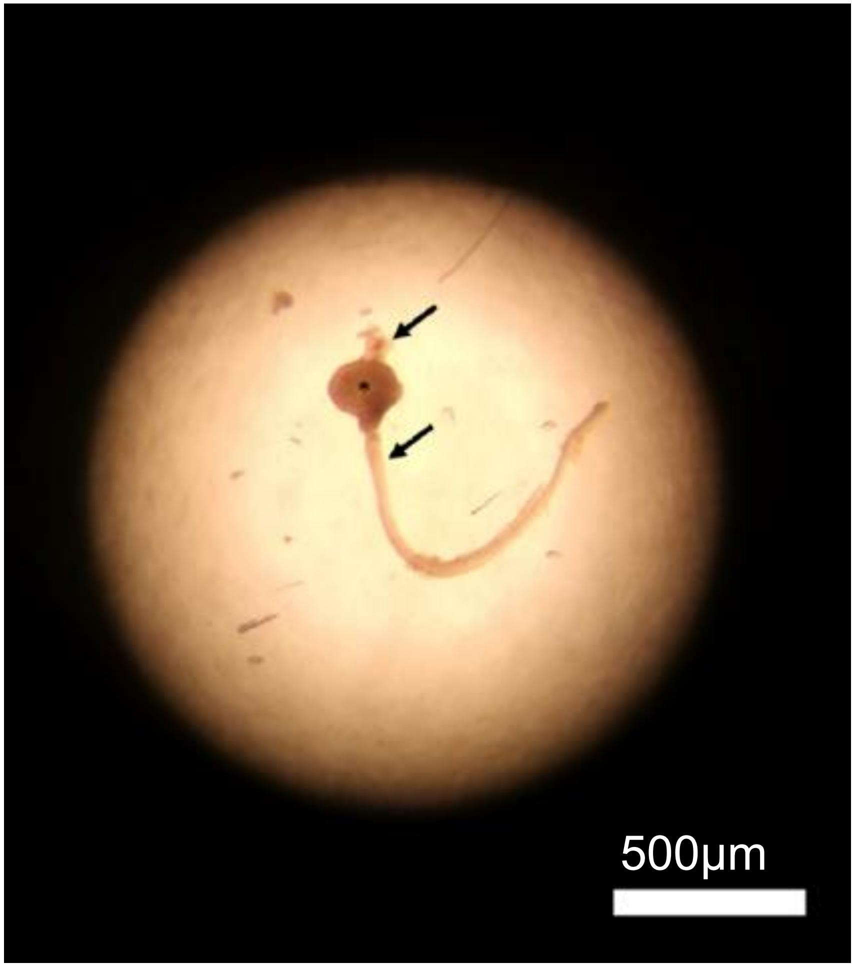
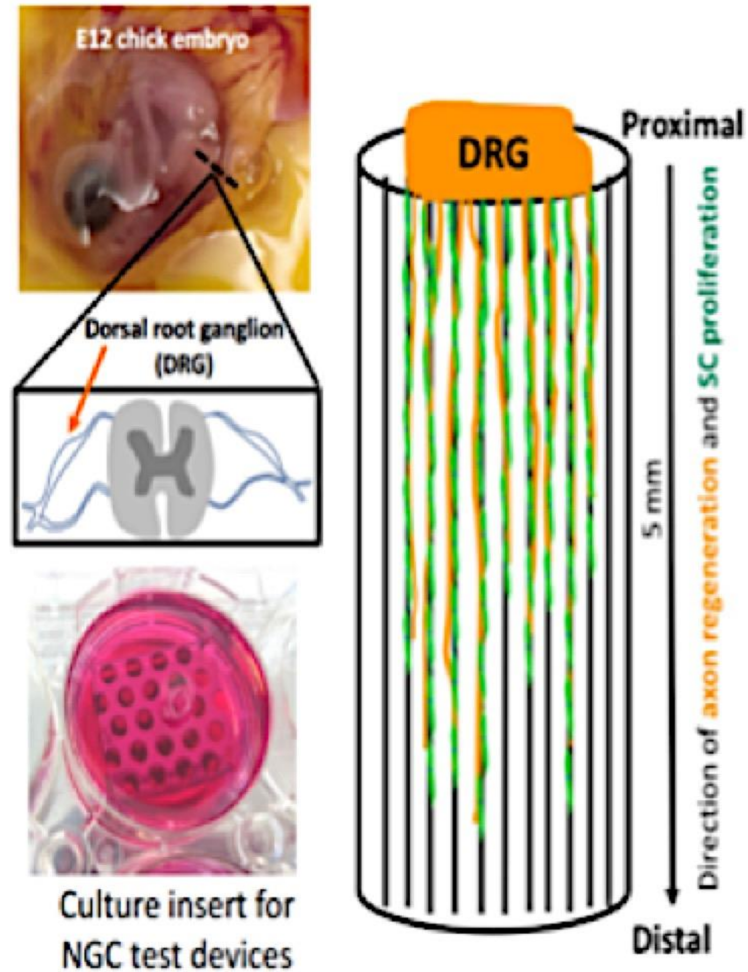
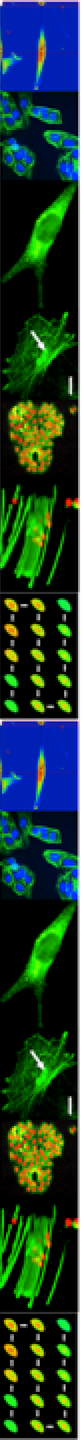
3D In vitro assessment model
E12 chick dorsal root ganglion







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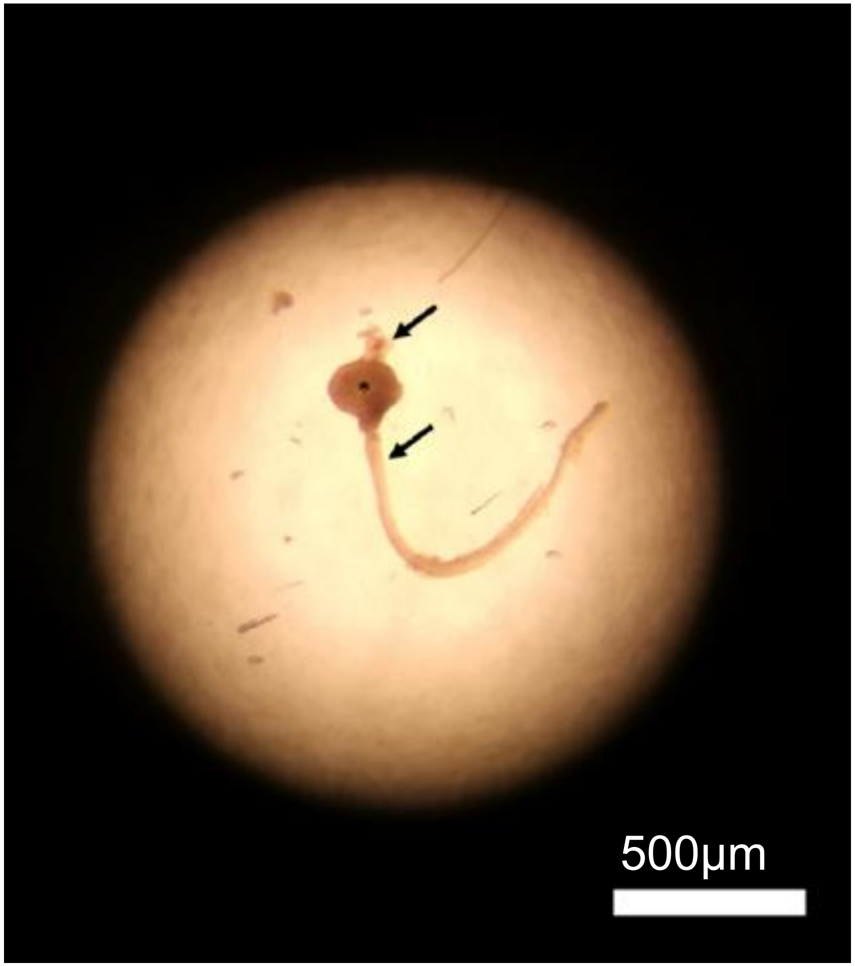

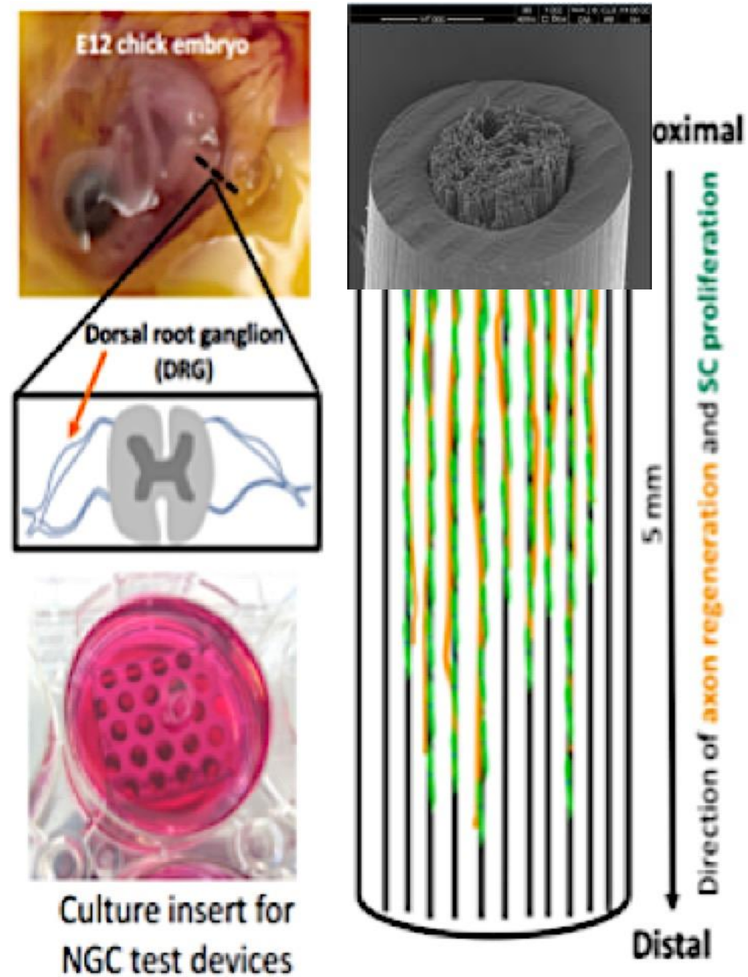

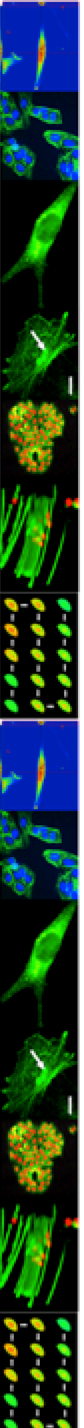

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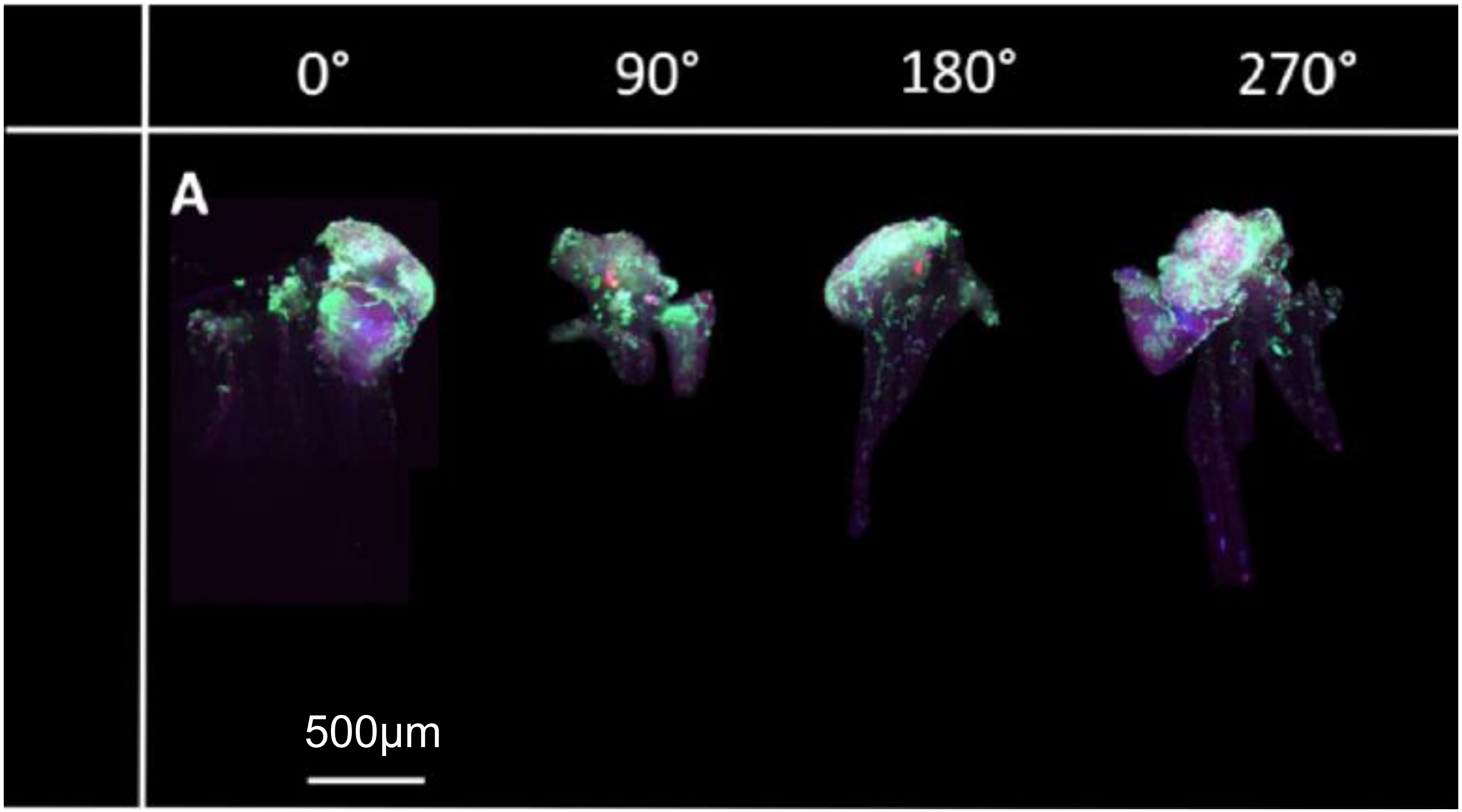

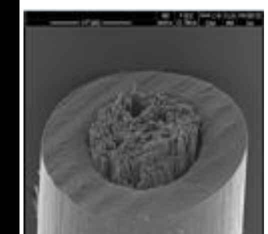

Light sheet microscopy (Zeiss Z1)

Blue = nuclei / Green = βIII tubulin / Red = S100β

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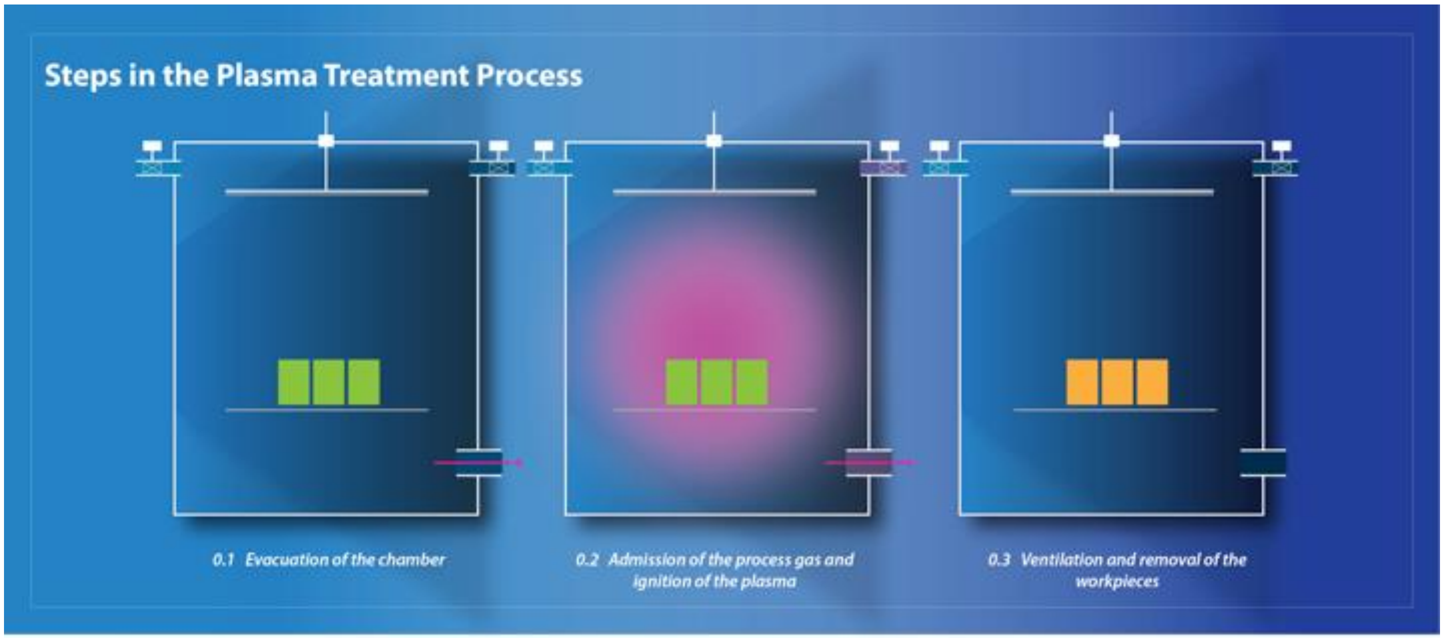
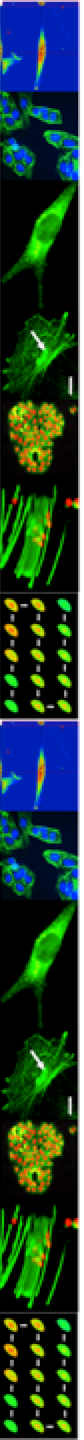
Surface modification of PCL fibres – air plasma



<u>Question</u> – Does air plasma surface deposition of PCL fibres change surface energy, elemental composition and adhesion / growth of neurons in a nerve guide device?

Diener Electronic commercial plasma system (model ZEPTO, chamber volume: 2.6L) with connected pump (Pfeiffer Vacuum Technology AG), 50 W (40 kHz) 0.4 mbar for 60 s.



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Air plasma surface treatment of PCL fibres X-ray photoelecton spectroscopy (XPS)

Sample	Wide scan (%)			Narrow scan						
				C1s region (%)					O1s region (%)	
	C	O	N	C-C	C-C-O	C(O)-O-C	COO	C=O	C=O	C(O)-O
FB - #1	79.6	20.5	0	45.1	12.3	12.6	9.7	0	10.5	9.9
FB - #2	82.8	17.2	0	50.3	13.3	11.6	7.6	0	9.4	7.8
FB - #3	79.3	20.7	0	40.6	14.8	13.6	10.2	0	10.2	10.6
FB + #1	78.1	17.6	0.9	29.5	25.1	13.1	5.6	4.9	10.6	7.0
FB + #2	78.4	17.8	1.2	29.3	26.1	13.2	5.5	4.4	10.8	7.0
FB + #3	79.1	16.8	1.2	29.8	26.7	13.3	5.4	3.9	10.2	6.6

+ = Plasma
– = No Plasma
 # = Position on the sample (1 = top, 2 = middle, 3 = bottom)
FB = fibre bundle

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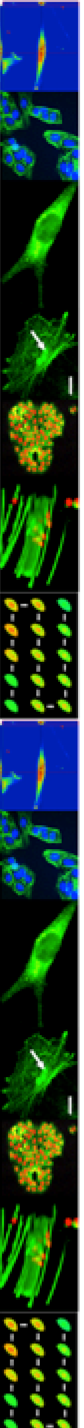
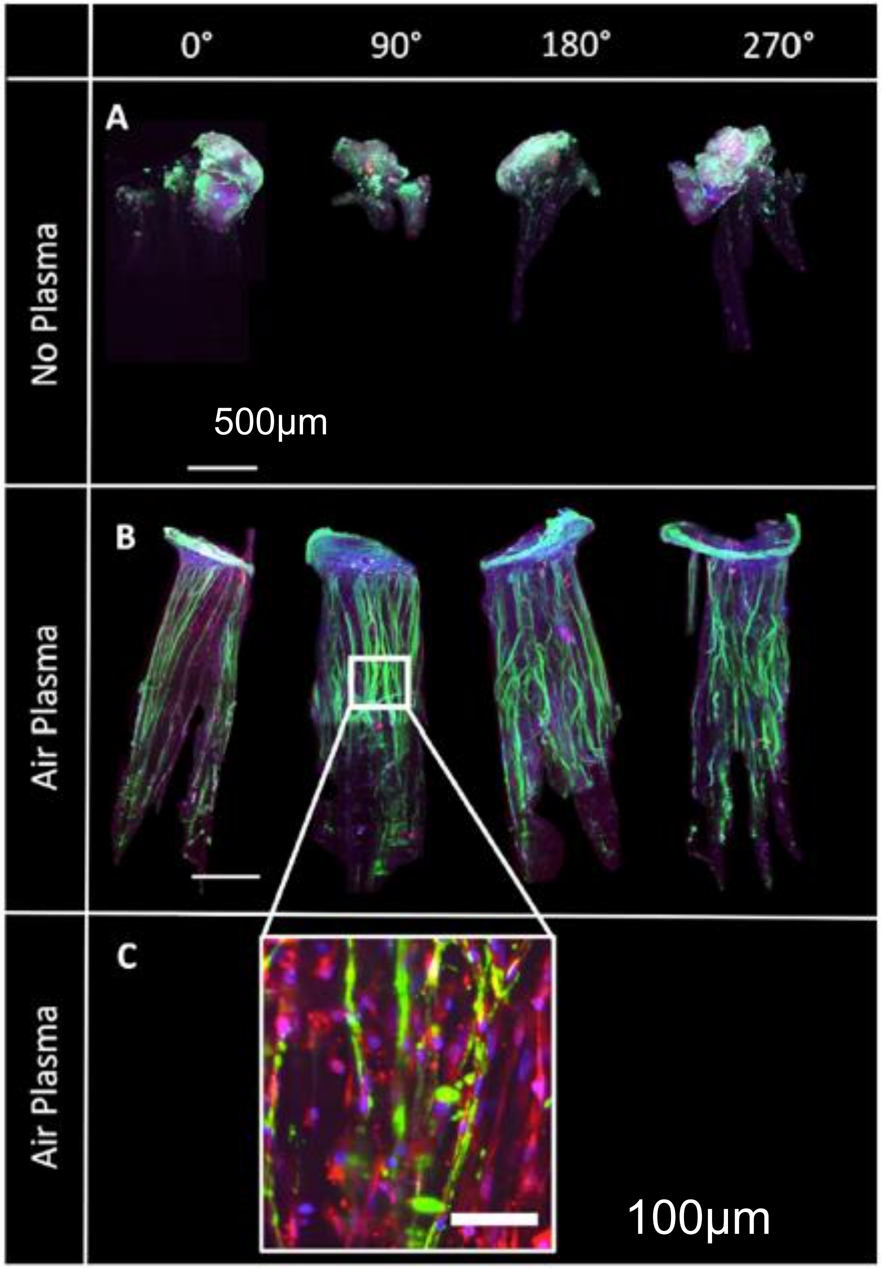
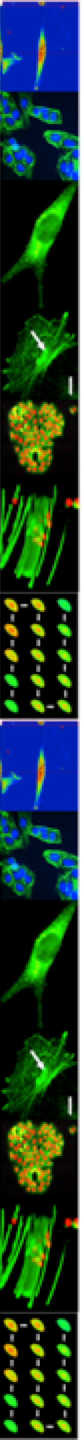




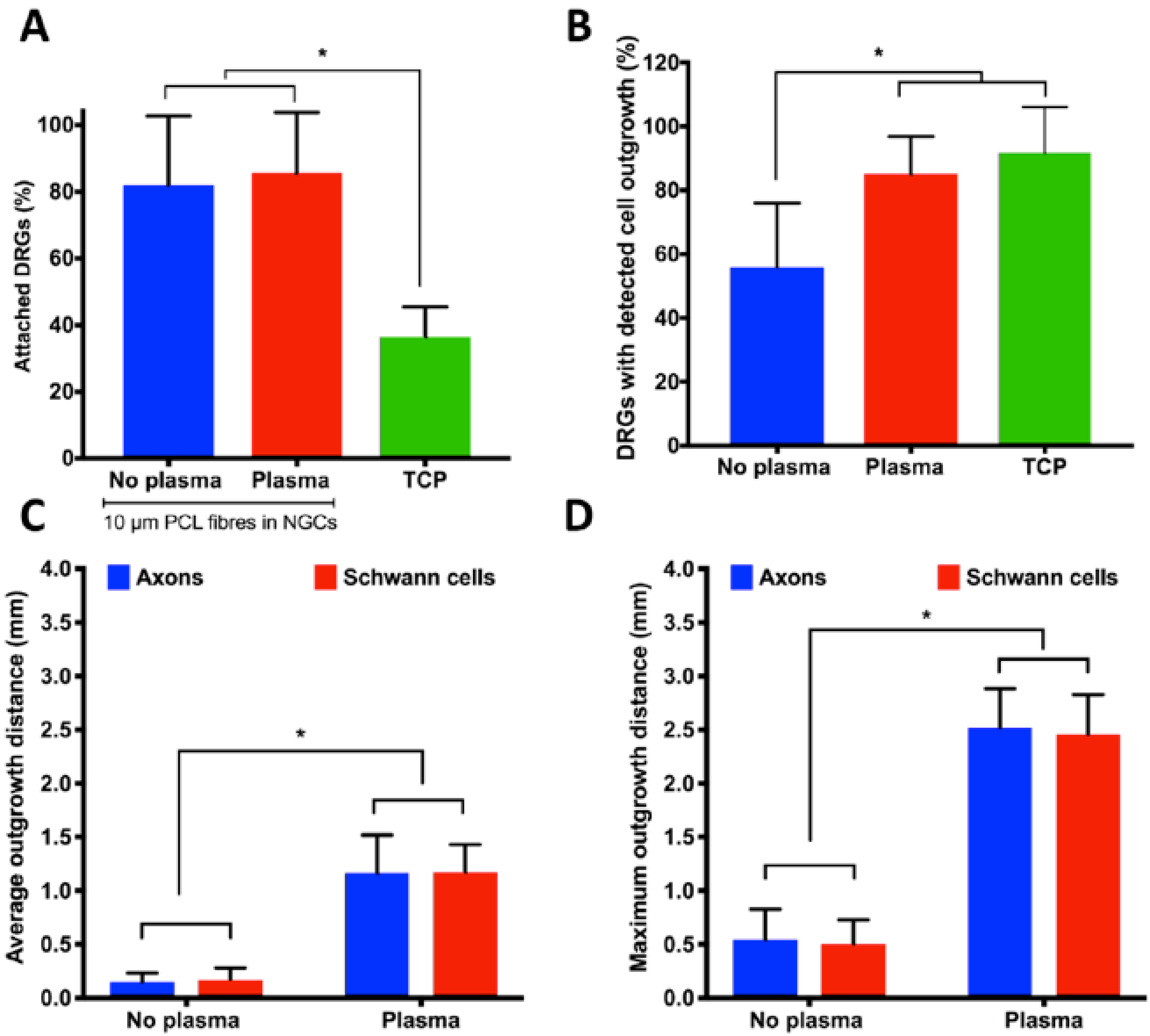
Air plasma surface treatment of PCL fibres E12 Chick DRG

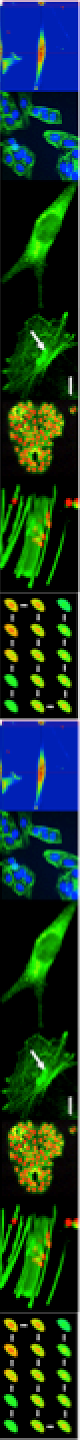
Light sheet microscopy (Zeiss Z1)

Blue = nuclei
Green = βIII tubulin
Red = S100β



3D In vitro assessment model
E12 Chick DRG





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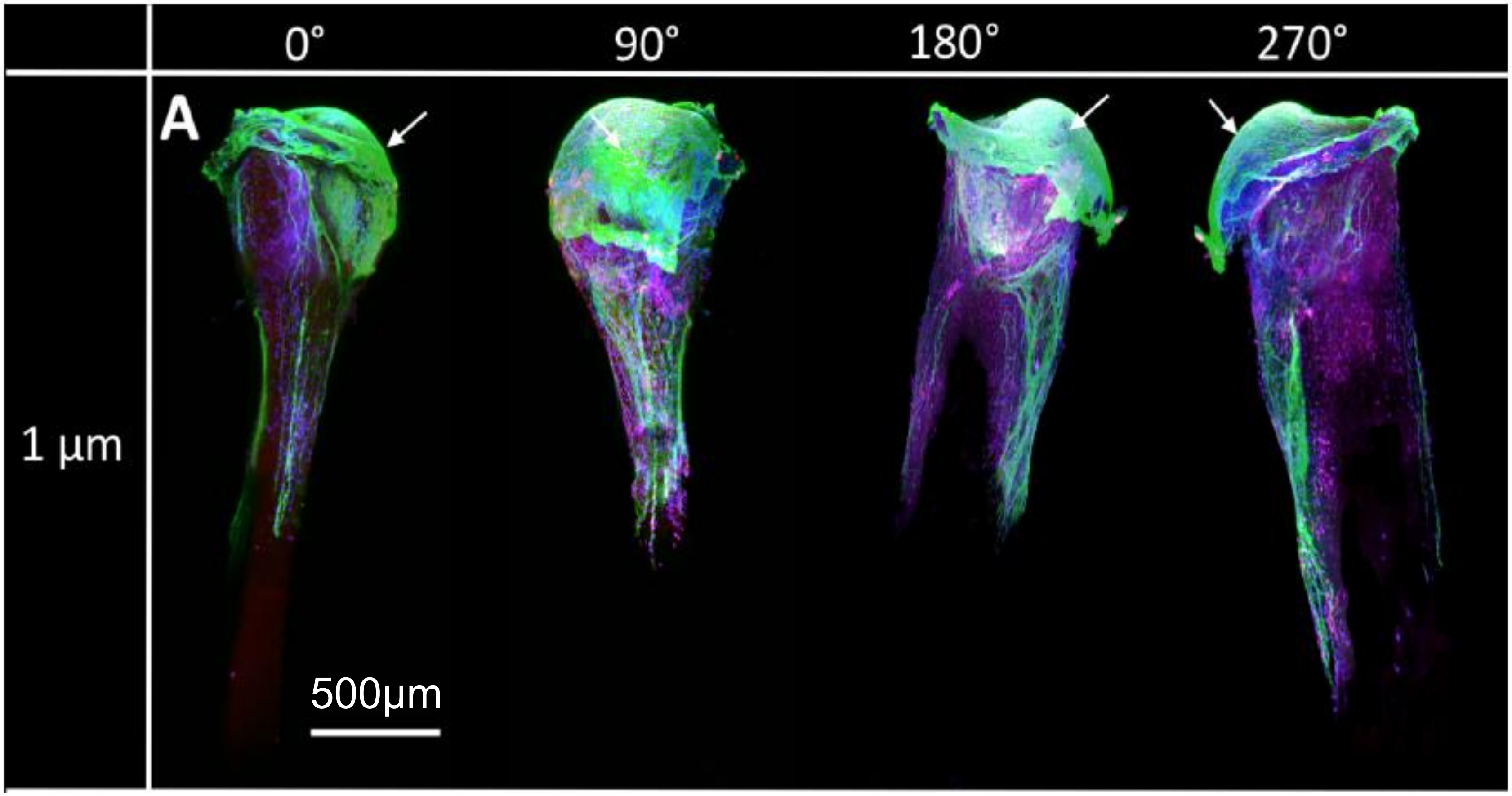
3D In vitro assessment model
E12 Chick DRG – PCL fibre diameter



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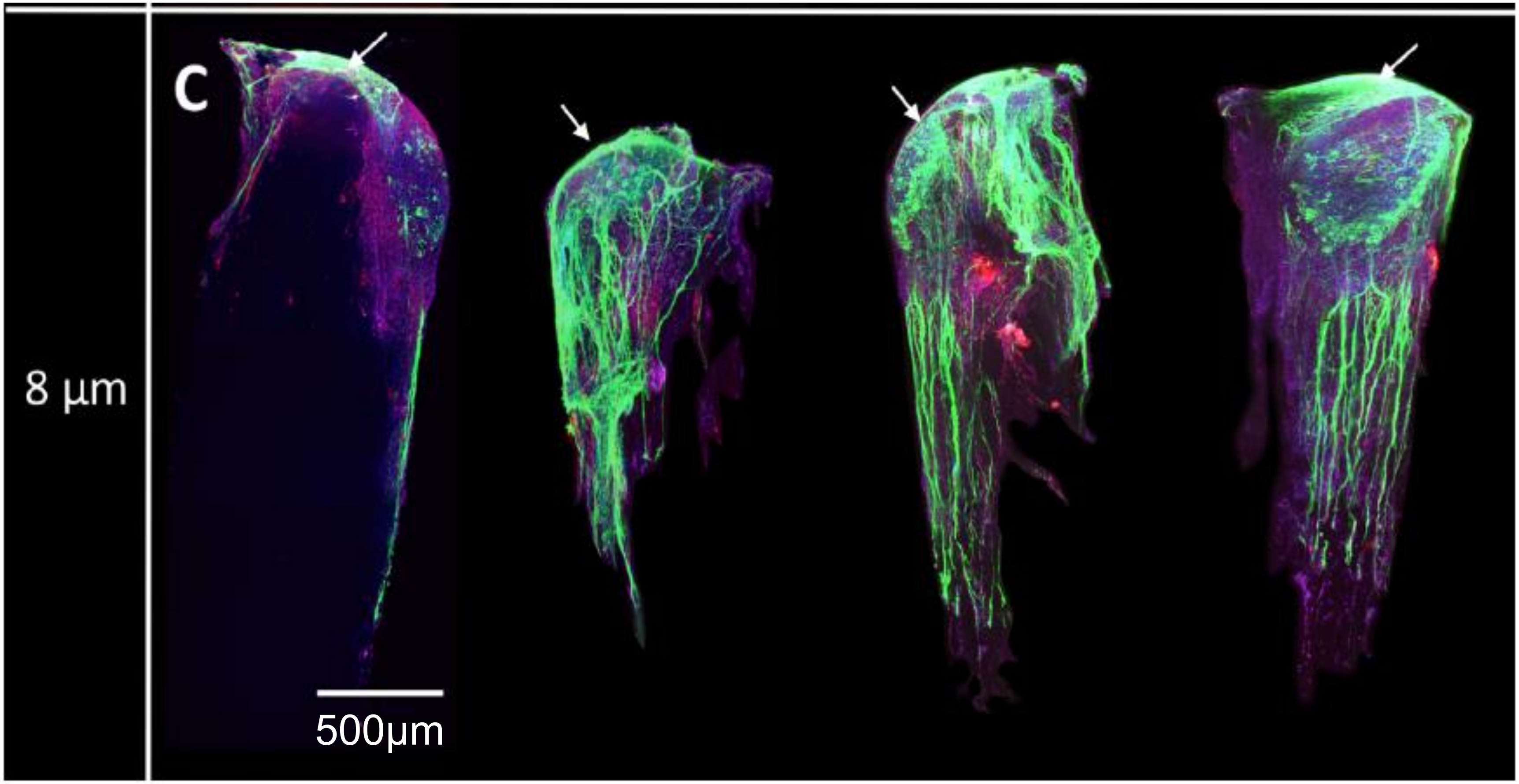
E12 Chick DRG – PCL fibre diameter



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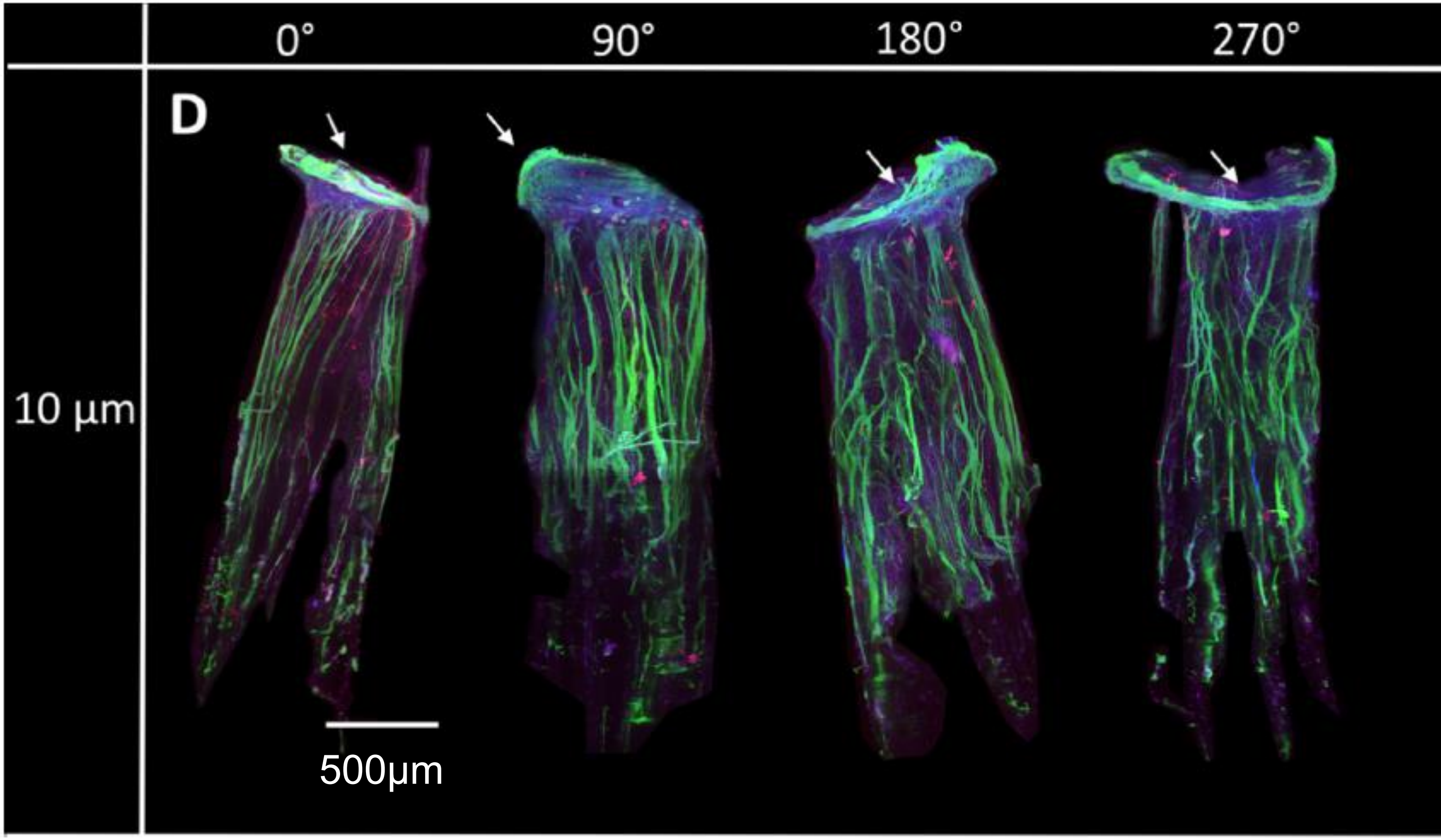
E12 Chick DRG – PCL fibre diameter



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3D In vitro assessment model
E12 Chick DRG – PCL fibre diameter

Light sheet microscopy (Zeiss Z1)
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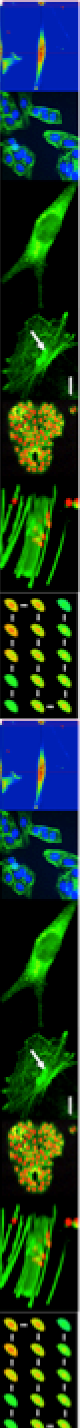
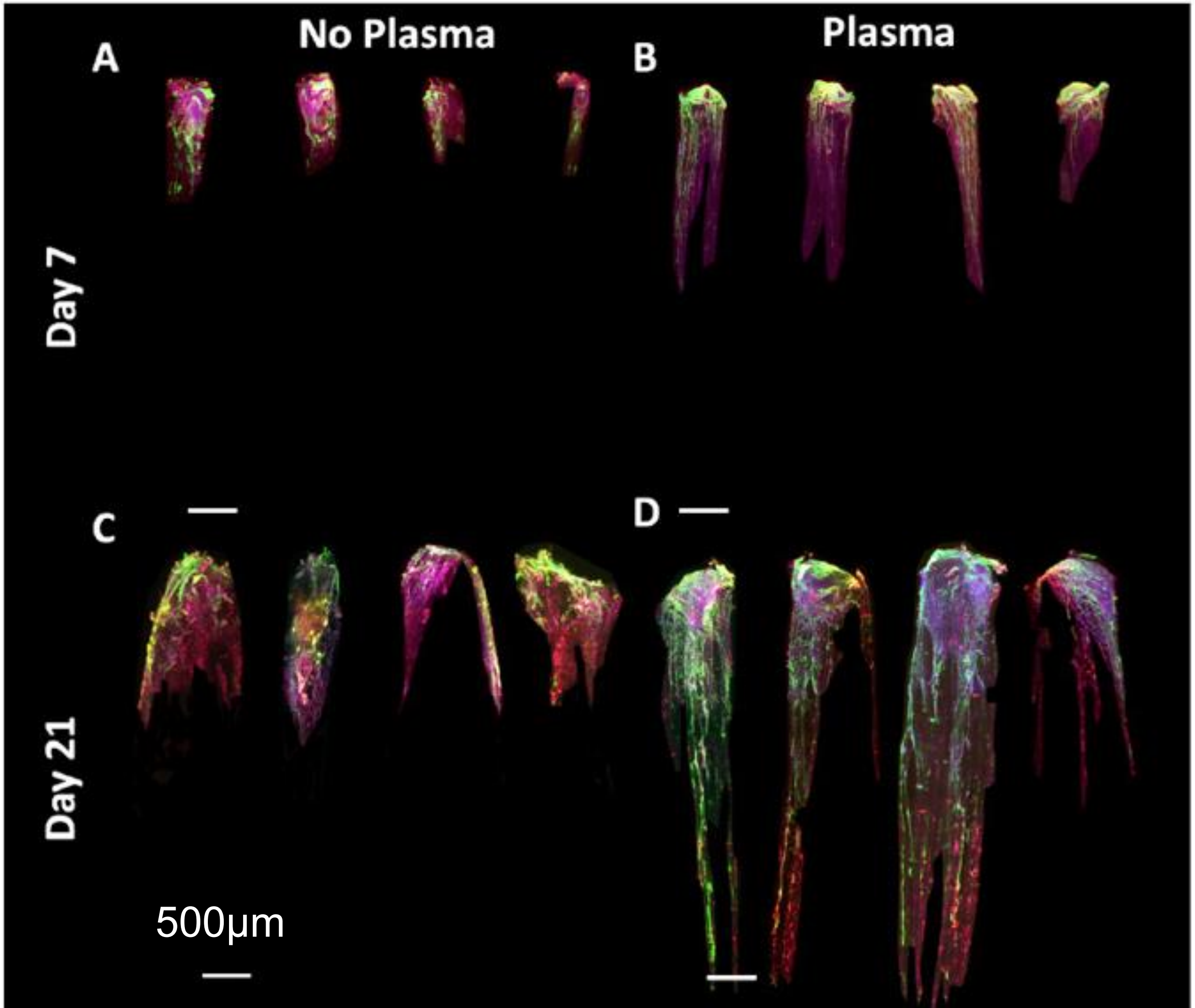
3D In vitro assessment model
E12 Chick DRG – 7d and 21d (10µm fibres)





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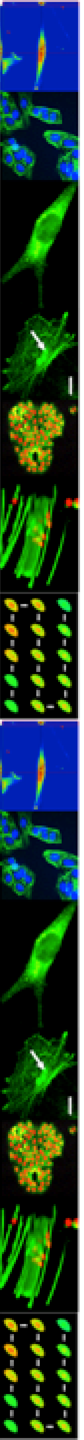
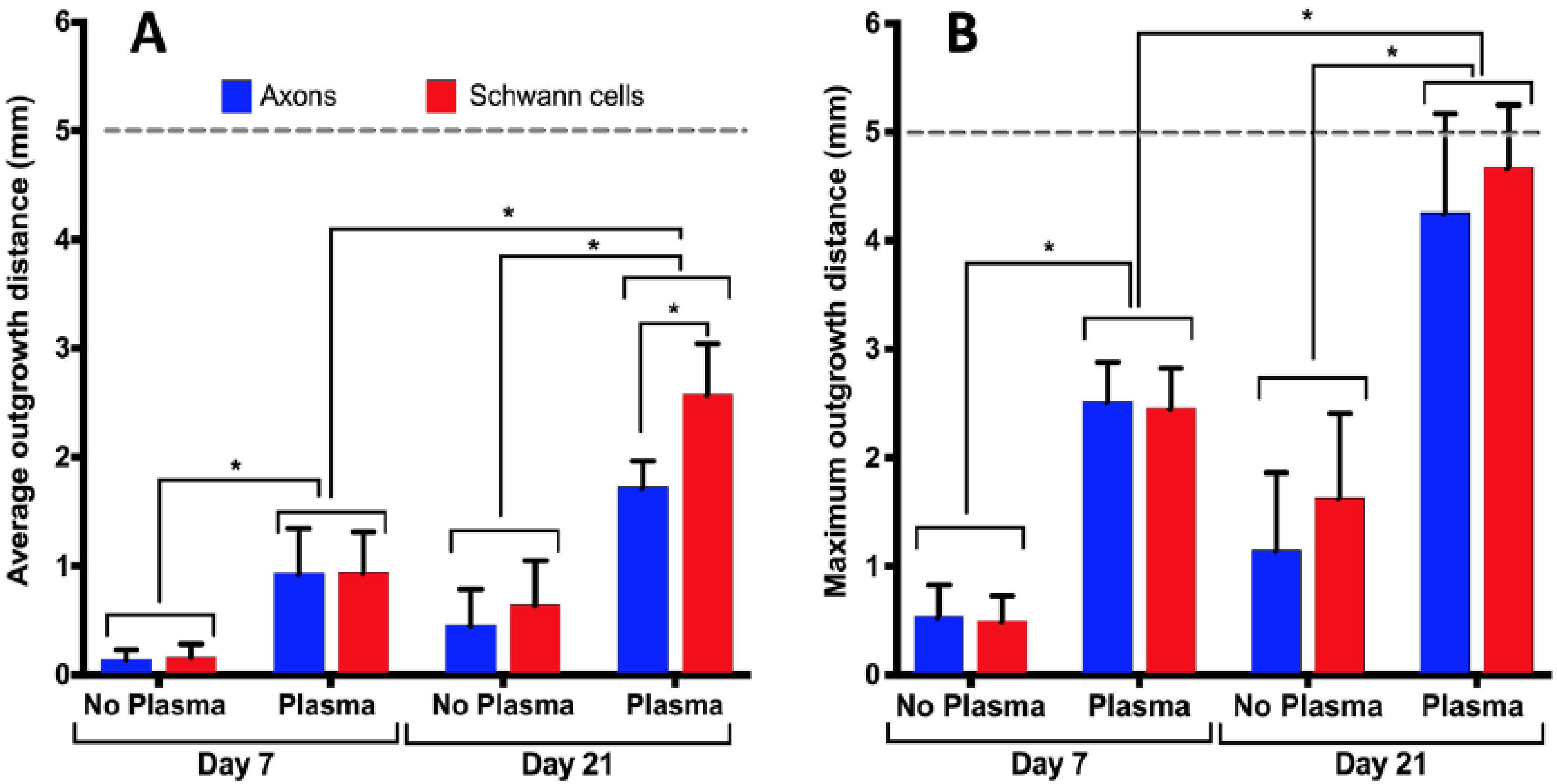
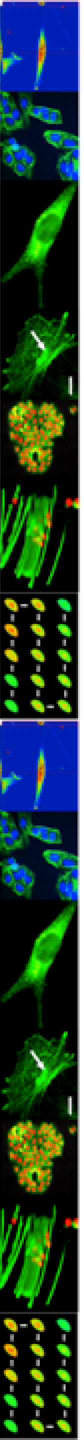
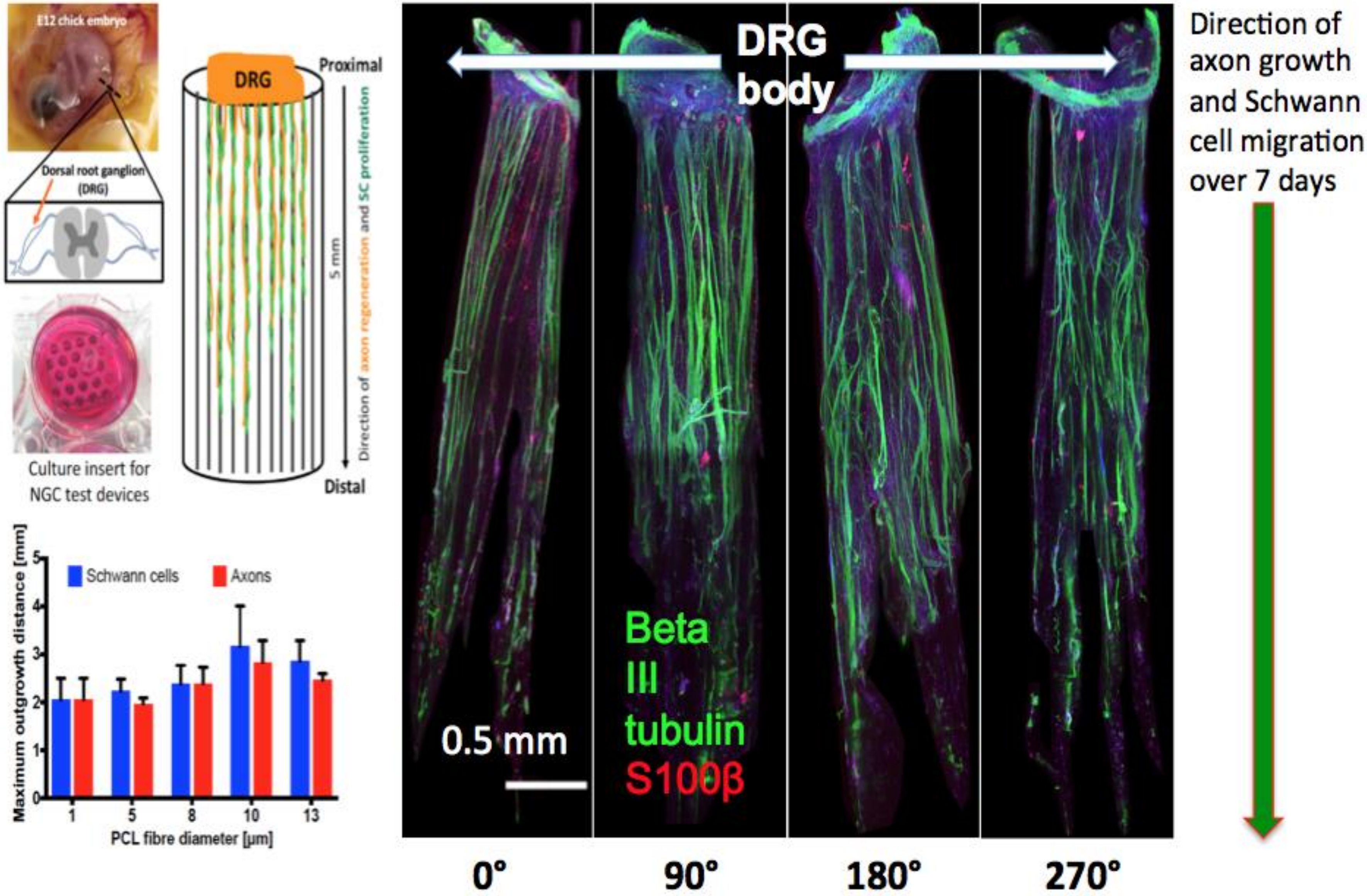
3D In vitro assessment model
E12 Chick DRG – 7d and 21d

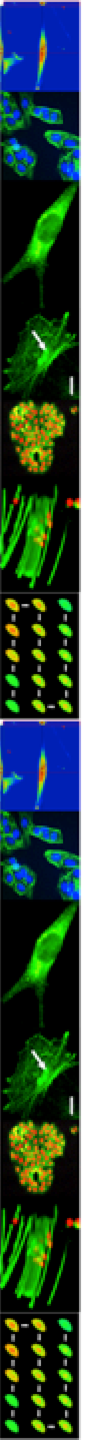






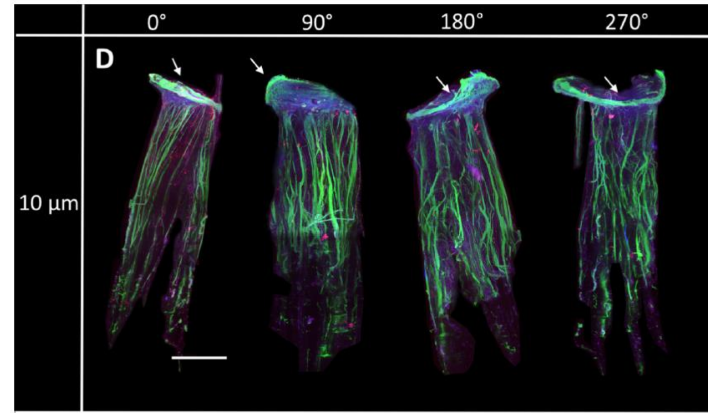


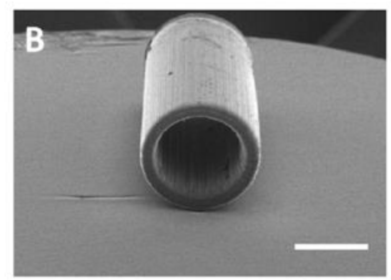
Behbehani M, Glen A, Taylor CS, Schumaker A, Claeyssens F, Haycock JW (2018) Pre-clinical evaluation of advanced nerve guide conduits using a novel 3D in vitro testing model. ***Int J Bioprinting*** 4(1) 1-12.

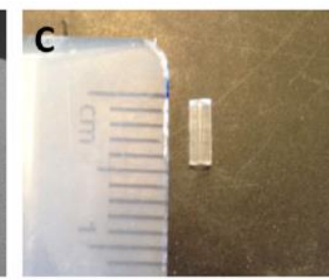


Conclusions

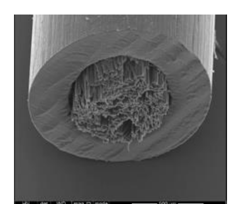
- 3D printed nerve guides made from PEG, and biodegradable PCL, PGS
- Support nerve repair *in vivo*
- Aligned internal fibre devices optimised
- Validated by microCT
- DRG models + light sheet
- Surface modification improves guided nerve growth

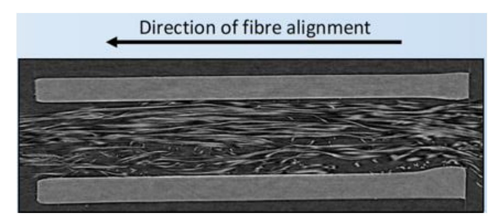


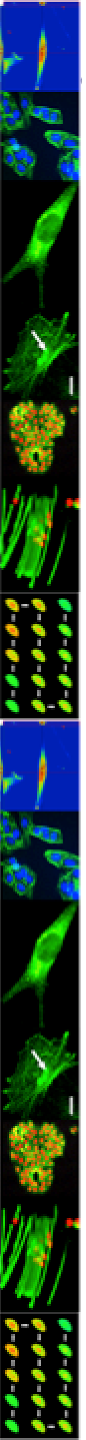




- Natural material NGC programme funded by Pak-UK fellowship IRC-BM / COMSATs – Dr Ather Farook Khan
- Study completed and ready to file patent for translation + commercialization







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