Conducting Organic Materials for Engineering Tissue

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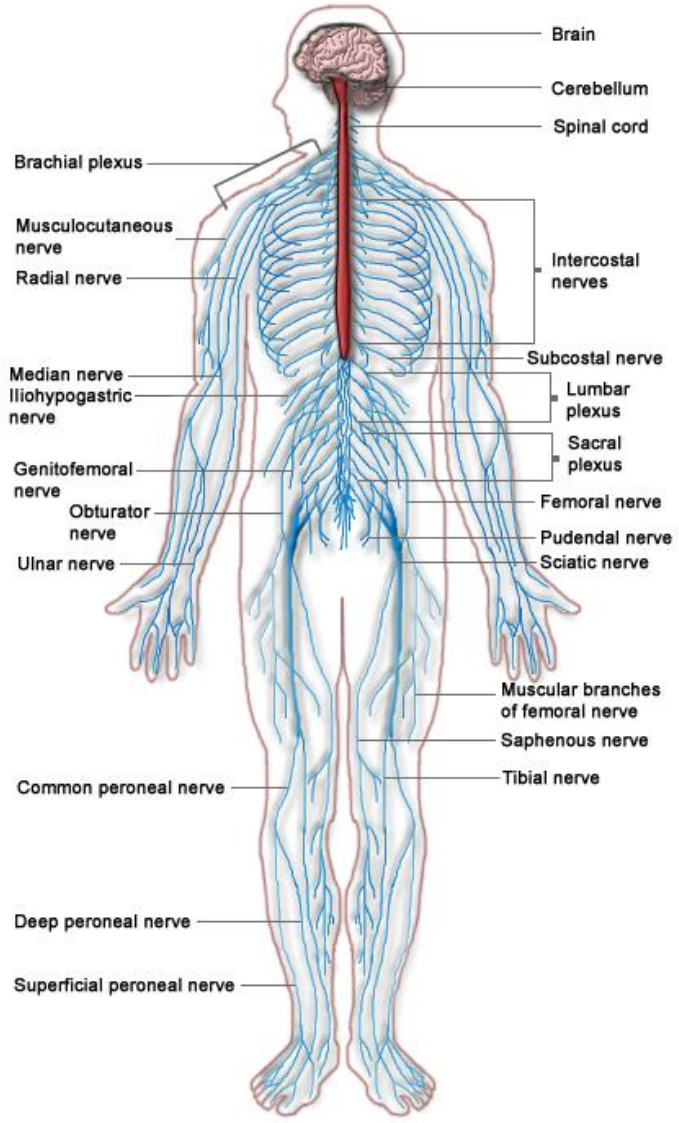
14/02/14

Content

- Conducting polymers and Nerve Regeneration,
  - Testing materials for biocompatibility
    - Conductivity and Contact angle measurements
    - In vitro testing, Cell adhesion, Cell toxicity
    - *in vivo* histology
- Functionalization of Polypyrrole using Carbohydrates aiming to create a new biomaterial conducting polymer,
  - 1,3 Cycloaddition Huisgen reaction CuAAC,
  - Characterisation of functionalised pyrrole NMR, C13, Dept, Mass spec.,
- Electrospinning
  - What is electrospinning?
  - The set up and materials
  - Types of applications

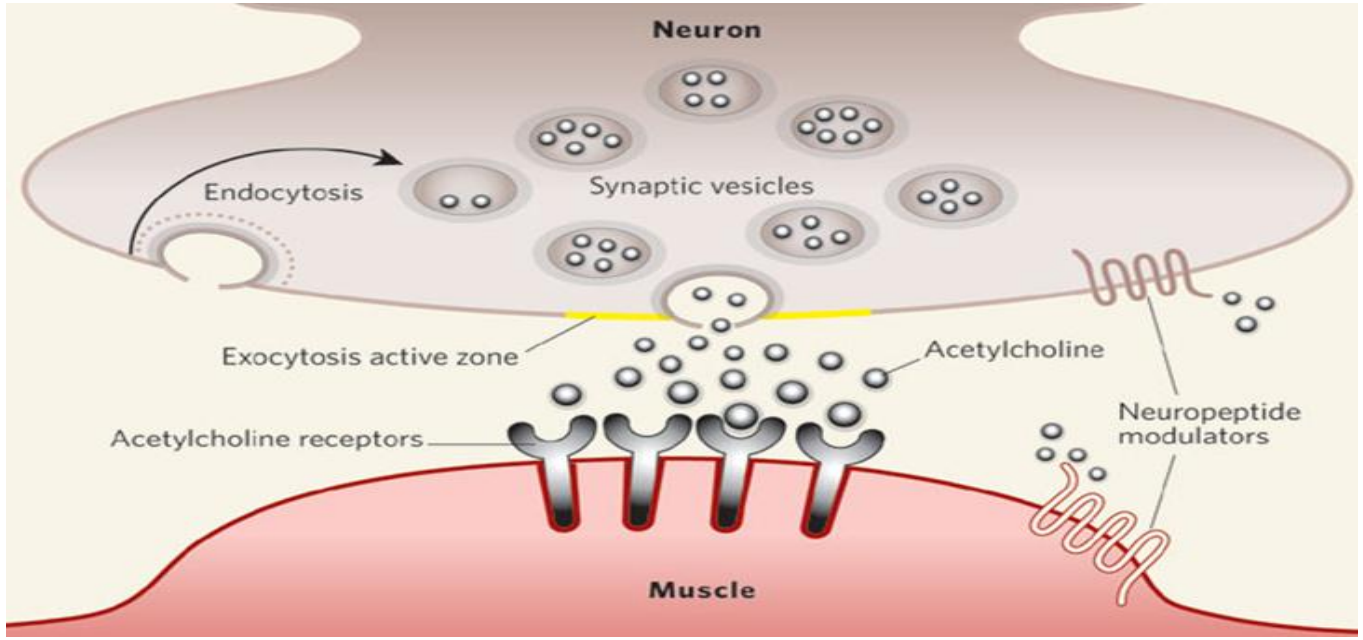
Peripheral Nervous System

- **Peripheral nervous system (PNS)**
  - all the nerves in your body, aside from the ones in your brain and spinal cord.
  - It acts as a communication relay between your brain and your extremities



1. http://upload.wikimedia.org/wikipedia/commons/b/ba/Nervous_system_diagram.png

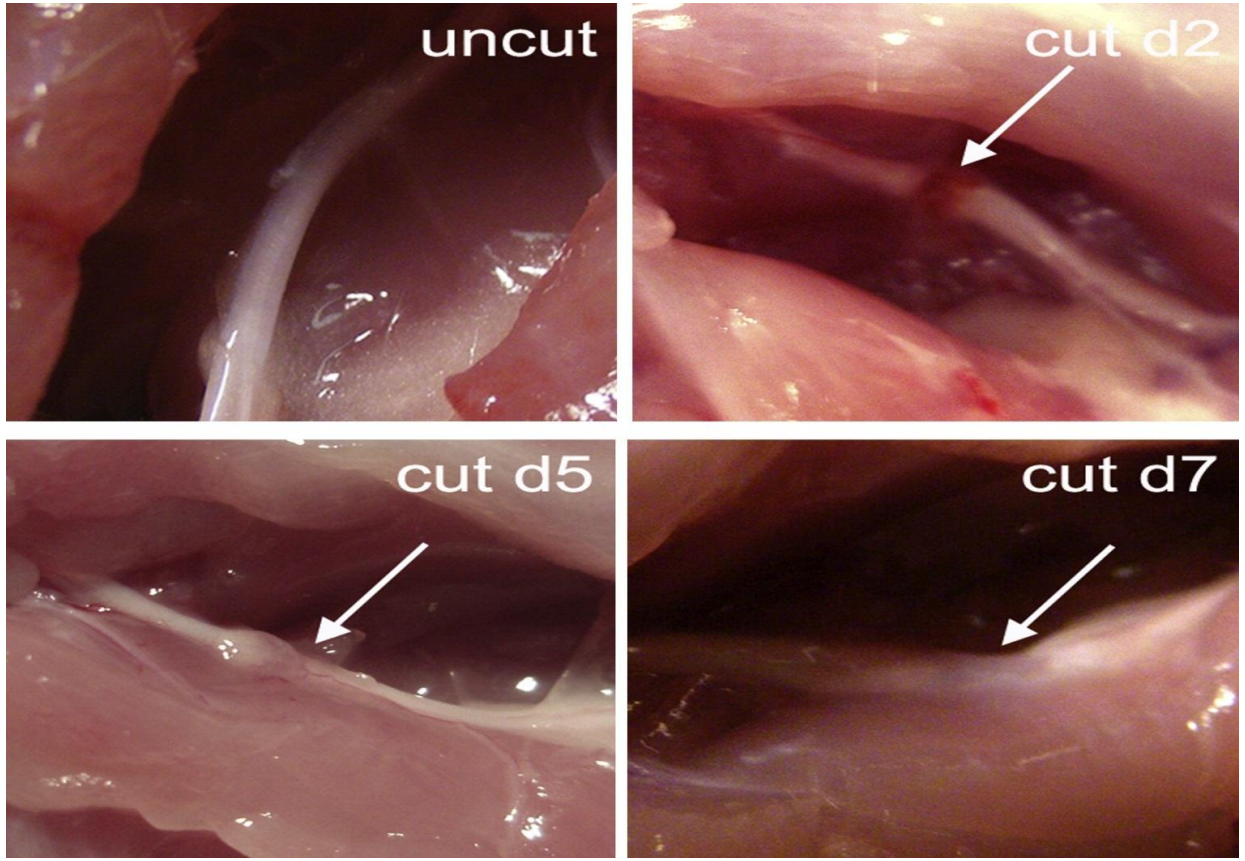
Muscle Contraction



Mechanism of Muscle Contraction

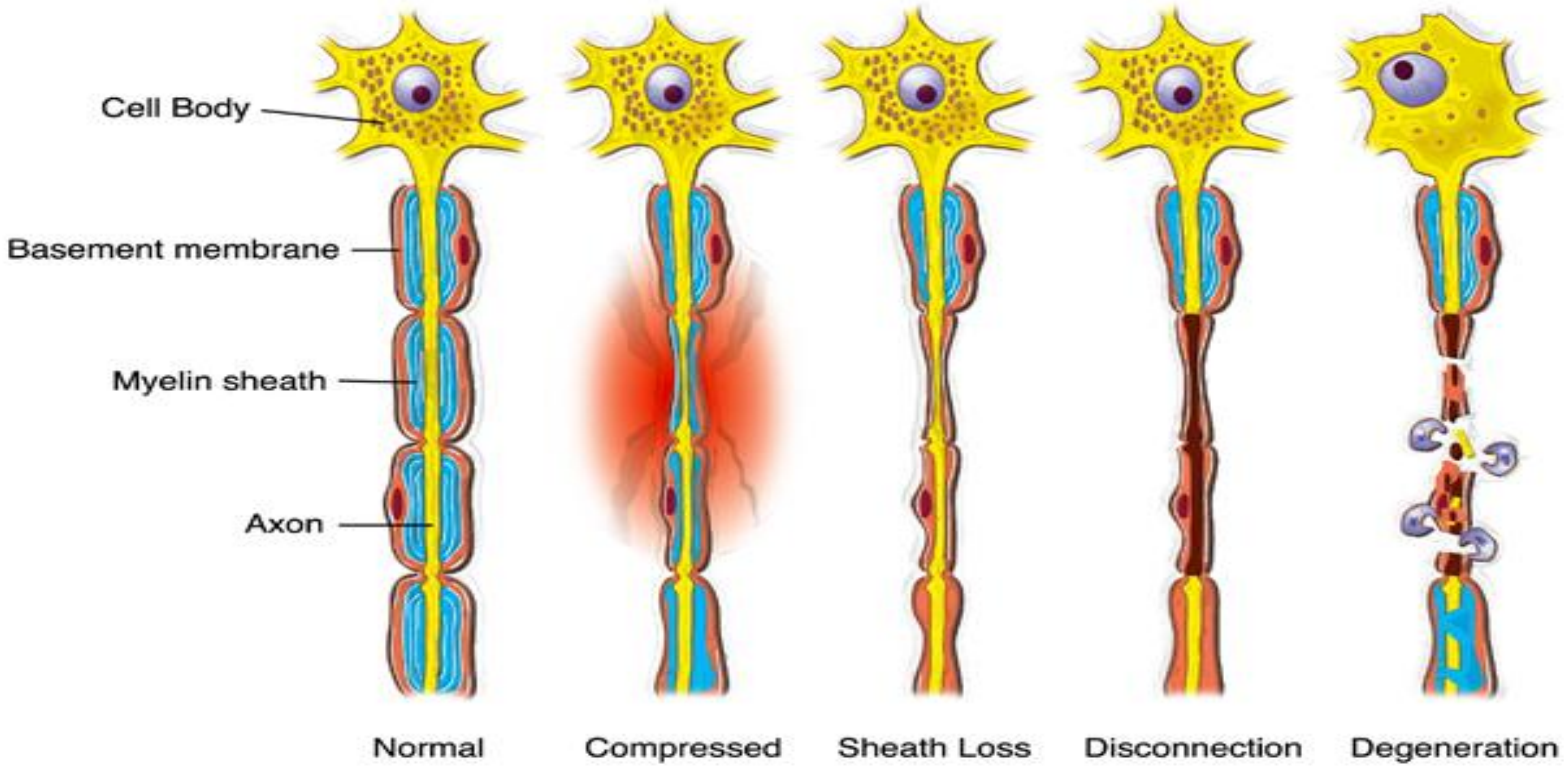
8. NATURE|Vol 436|28 July 2005

Peripheral nerve regeneration



- Nerves will regenerate
- 1 mm a day
- Depending on the severity of the injury

Nerve Injury

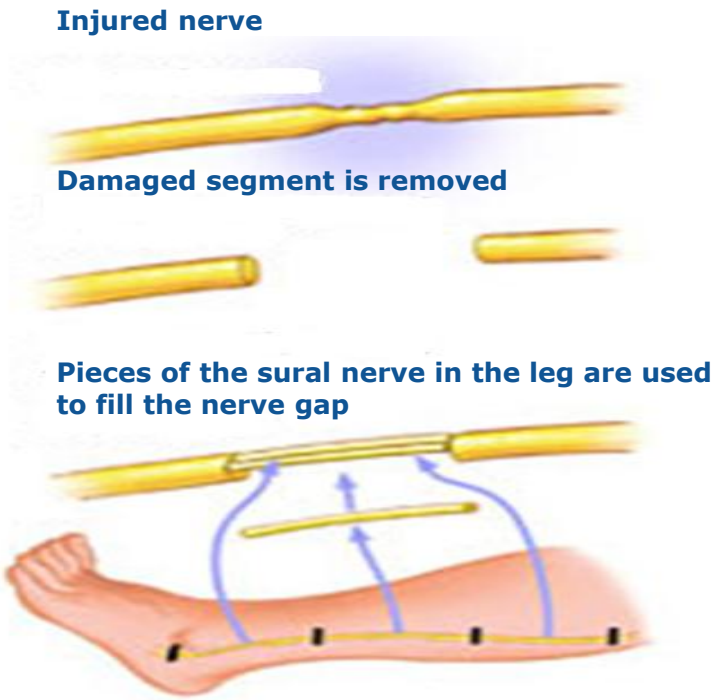


→ **Muscle Atrophy**

2. http://www.backpain-guide.com

State of the Art

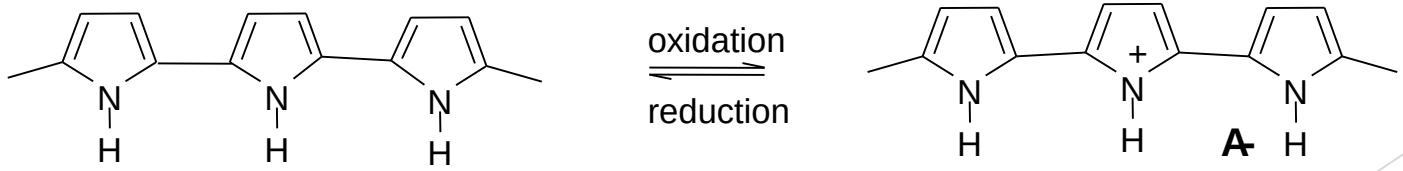
- Autologous nerve grafts
  - Limitations
    - Multiple surgeries
    - Loss of function at donor site
- Nerve Conduits
  - Hollow Collagen tubes
  - Nerves need guidance
- Regeneration is slow



http://www.mayoclinic.org/images/nerve-graft-1-162-bdy.jpg

Polypyrrole

- Polypyrrole (Ppy) is a conducting polymer, an organic material comprised simply of C, H and simple N heteroatoms
- By incorporating anions (doping) into the polymer during synthesis the resistivity is greatly reduced giving it conductivity
- Polypyrrole has been shown as a material for the delivery of a number of bioactive compounds including Neurotropic factors.[5]
- Previously shown to release dopamine so has the capability of releasing other growth factors and NTs such as Acetylcholine



PPy^{0}

Neutral – reduced
Release of Drug

$PPy^{+}\ A^{-}$

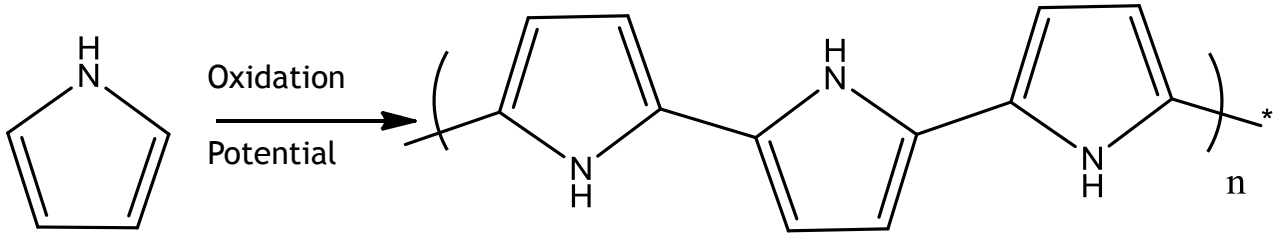
Oxidized state
anion doping and
binding of Drug

Conducting polymers and Electrical stimulation

- Promising new approach combining scaffolds with electrical stimulation
- Recent literature has shown that electrical stimulation enhances the rate of nerve regeneration.[6]
- Mechanism is unclear but some believe the electrical stimulus alters the local electrical fields of ECM molecules changing protein adsorption.[7]
- Electrical stimulation as a new therapeutic approach to accelerate nerve regeneration after injury and improve functional recovery
- Lack of suitable conducting materials
  - Ppy is promising due to it's conductivity, biocompatibility and its ability to undergo surface modification with bioactive agents

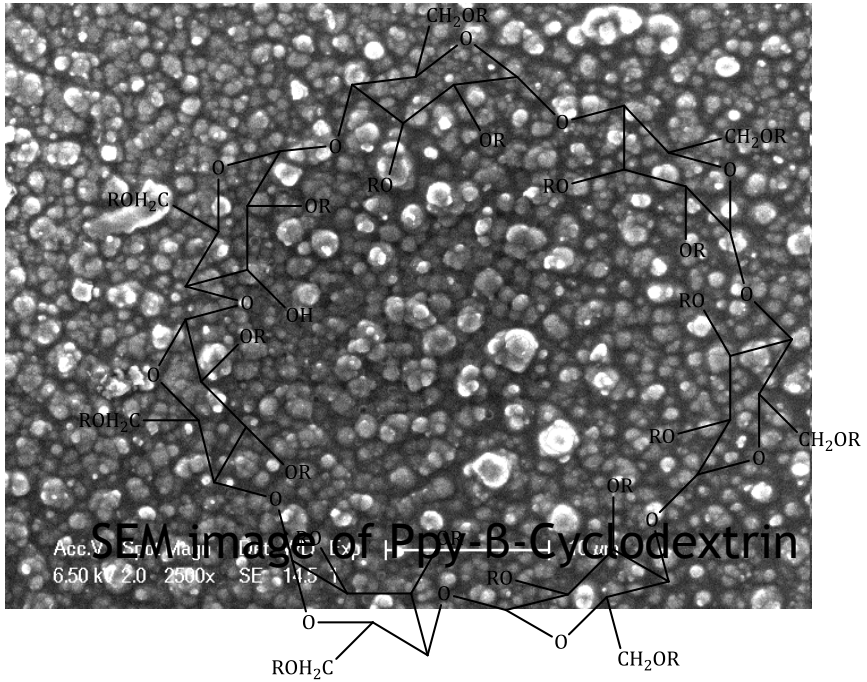
5. *Biomaterials, Volume 32, Issue 15, May 2011, Pages 3822-3831*
6. Biomaterials. 2001 May;22(10):1055-64.
7. Proc. Natl. Acad. Sci. USA 1997 August; 94, 8948–53

Materials and Methods

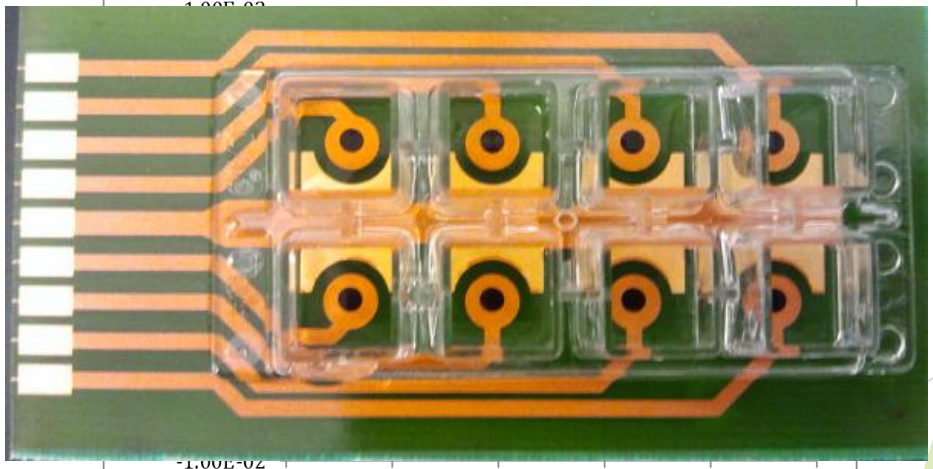


Pyrrole

Polypyrrole

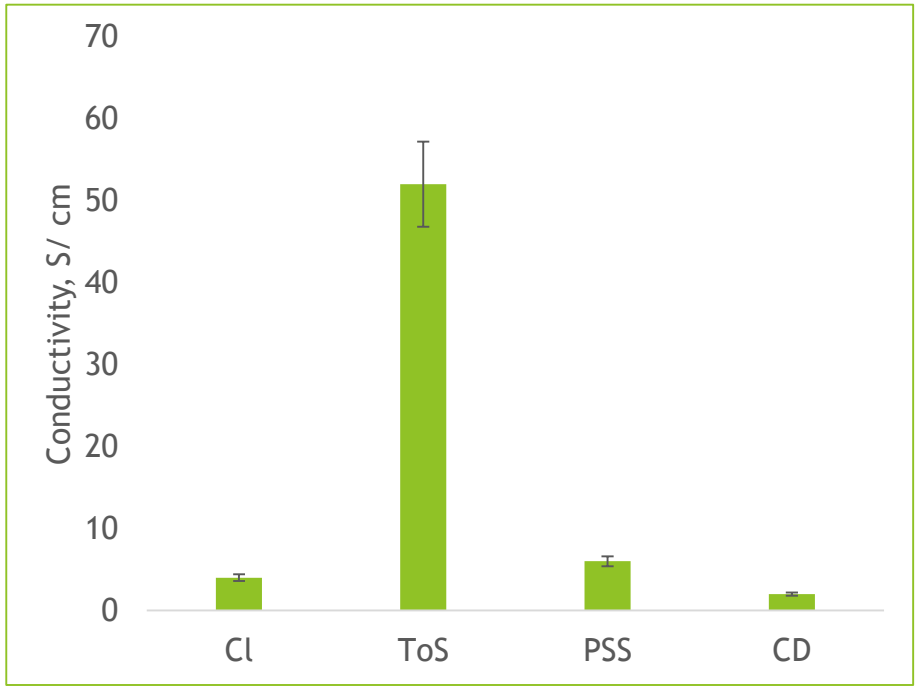


SEM image of Ppy-β-Cyclodextrin



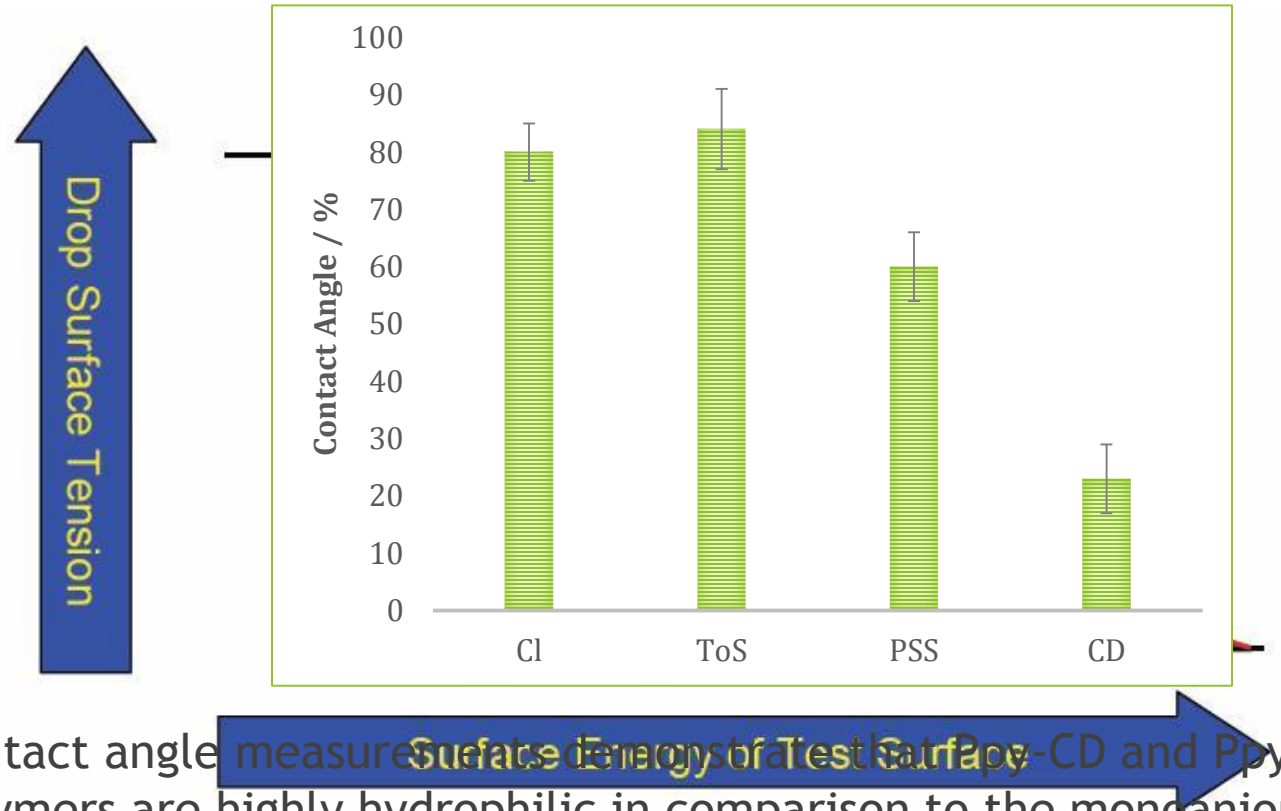
ECIS cell culture plate

Conductivity



- Difference in conductivity most likely relates to the entrapment of a proportion of CD ions that do not actually dope PPy. For a CD molecule in a PPy film, steric hindrances only allow a fraction of the anions on the molecule to contribute to the conductivity of the film.
- ToS ions, in contrast, are able to move more freely to allow stronger doping interactions with PPy.
- The small size and high solubility of chloride make it a less stable dopant, and even washing may have disrupted the doping interactions, resulting in de-doping and thus less conductive Cl-doped films.

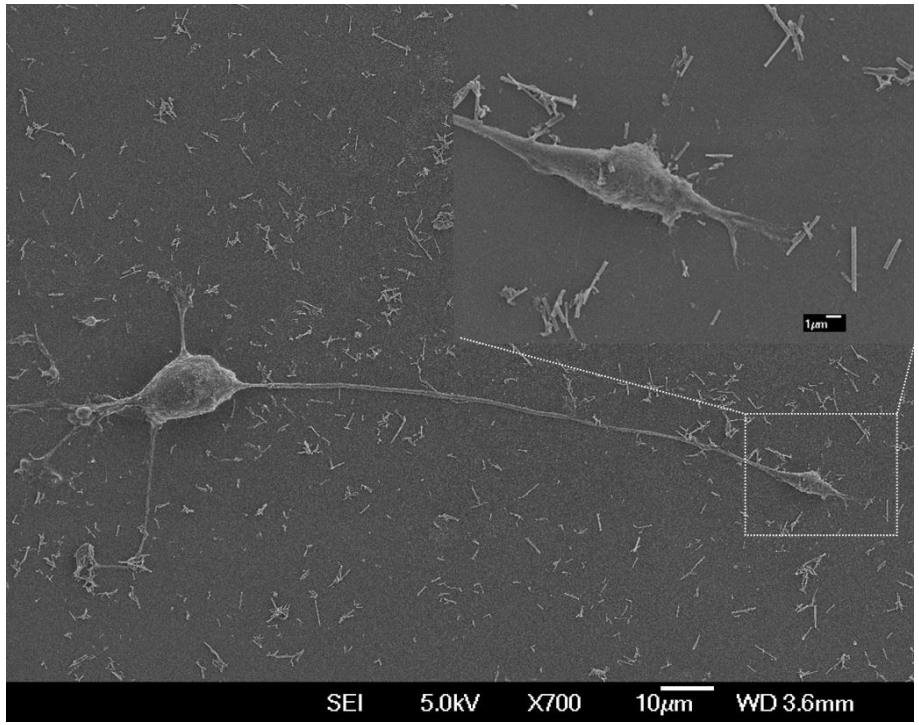
Contact Angle



- Contact angle measurements demonstrate that Ppy-CD and Ppy-PSS doped polymers are highly hydrophilic in comparison to the monoanionic doped Cl and ToS polymers
- Due to the free charges being present in the CD and PSS systems over the no free charges in the Cl and ToS polymers

http://www.astp.com/archives/11383

PC12 cell line



- PC12 is a cell line derived from a pheochromocytoma of the rat adrenal medulla
- PC12 cells stop dividing and terminally differentiate when treated with nerve growth factor making them a useful model system for nerve regeneration.
- They are an adherent dependent cell line

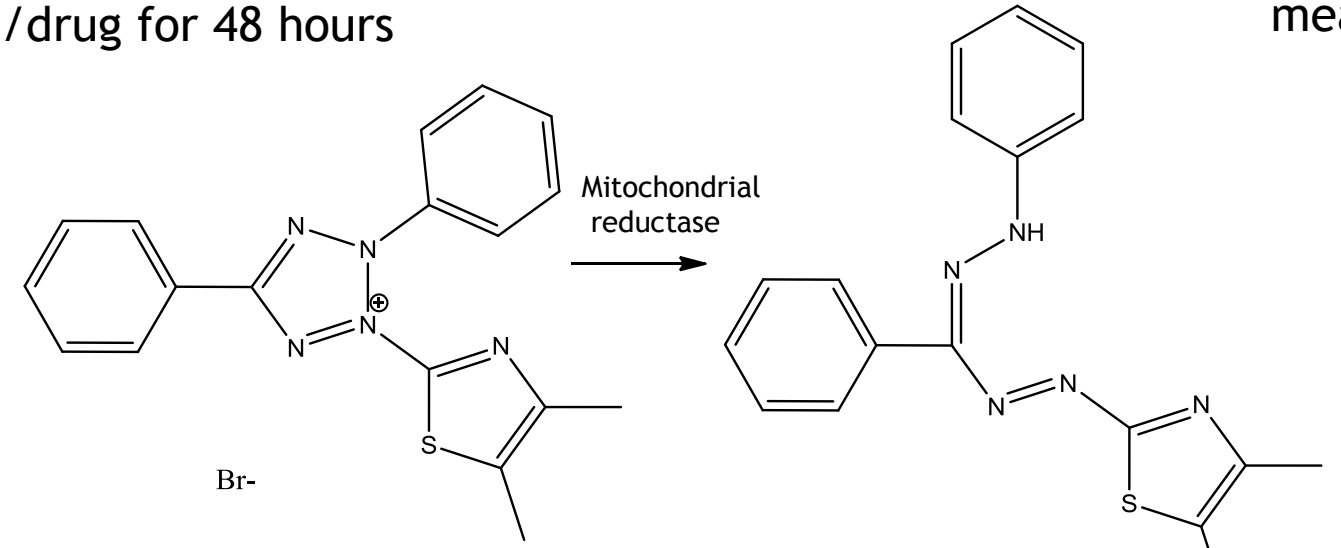
Cell toxicity – MTT assay

1: Seed cells then expose to material/drug for 48 hours

2. Add MTT

3. Incubate 4 hrs

4. Dissolve Formazan and measure Abs at 565 nm

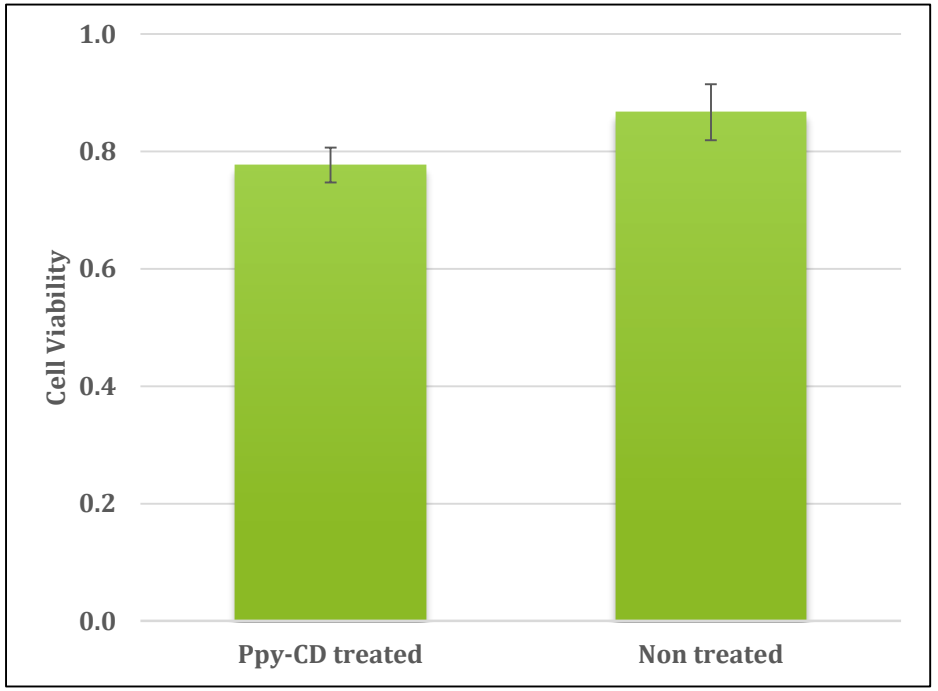


MTT (yellow)

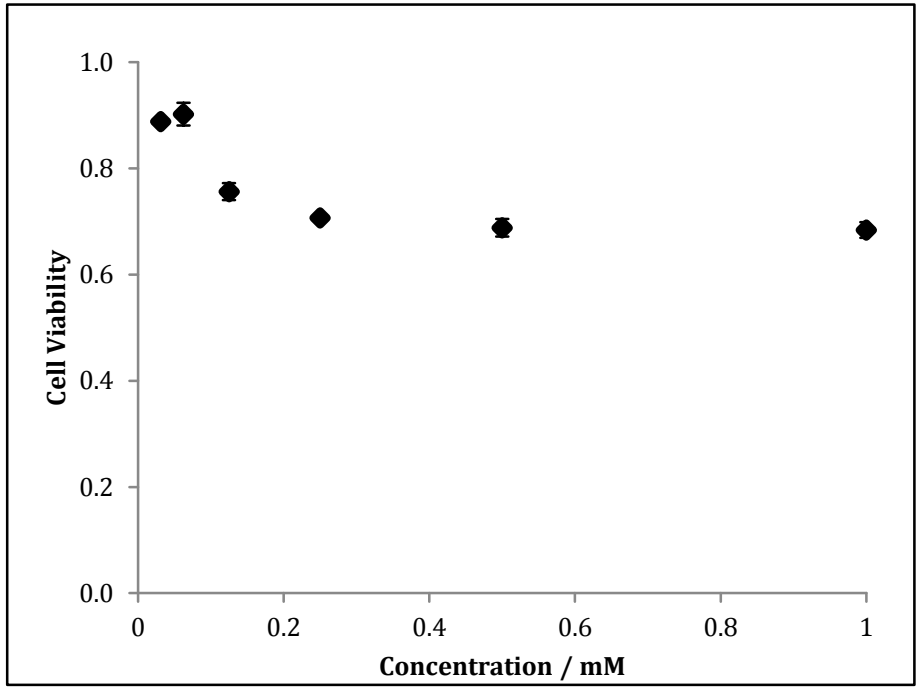
Formazan (purple/Blue)

Journal of lmmunologicalMethods, 65 (1983) 55-63

Results - Characterization



Material



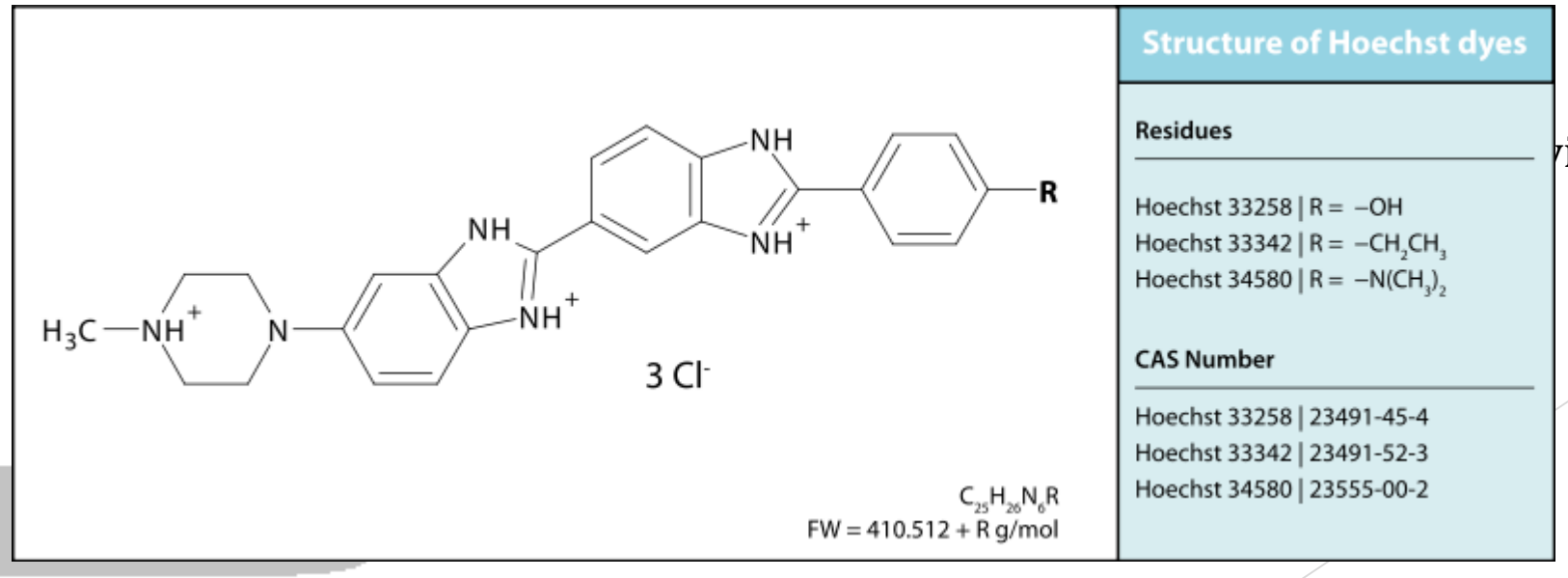
Cyclodextrin only

Cell adhesion

- Cell adhesion to polymer surfaces has obvious implications in the field of tissue engineering
- Facilitating cellular adhesion, growth and differentiation onto a surface can aid in wound healing and tissue growth
- A polymer can provide mechanical stability for the newly forming tissue

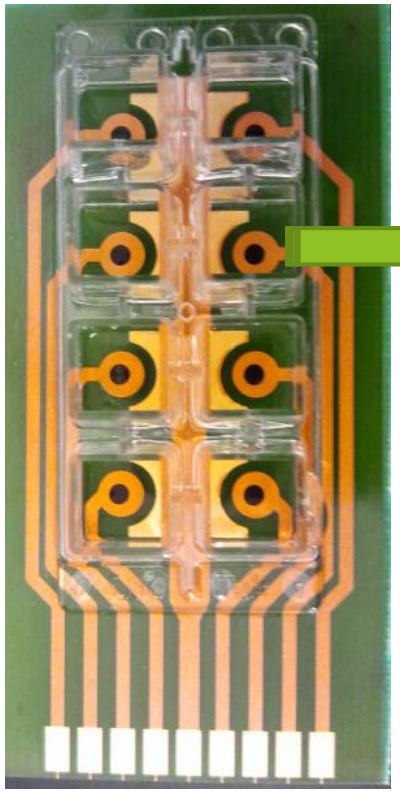
Cell Immunocytochemistry

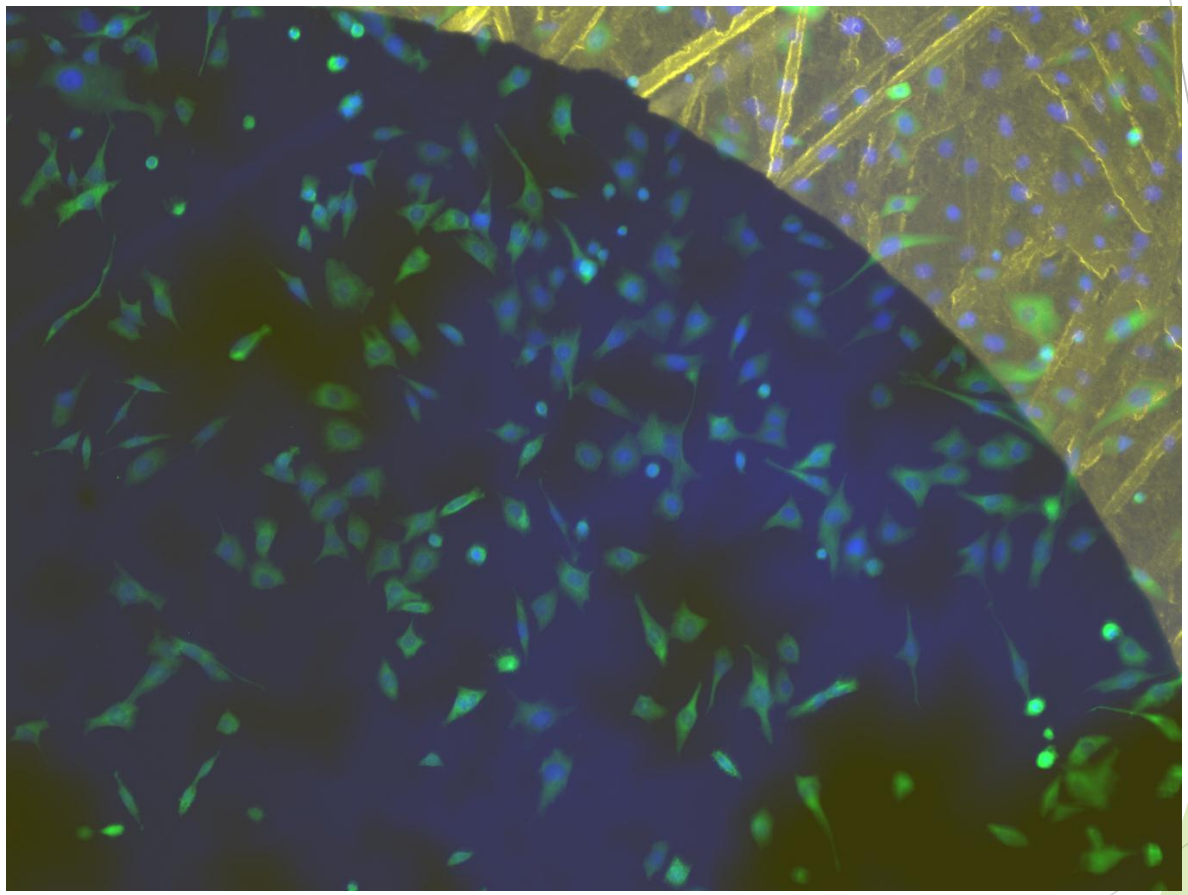
- Immunocytochemistry is a technique used to assess the presence of a specific protein or antigen in cells by use of a specific antibody that binds to it.
- The antibody allows visualization of the protein under a microscope.
- PC-12 use a tubulin antibody to adhere to the tubulin protein in the cell
- Hoechst a fluorescent stain that binds strongly to DNA was used to highlight the nucleus of the cell.



ith FITC

Cell adhesion



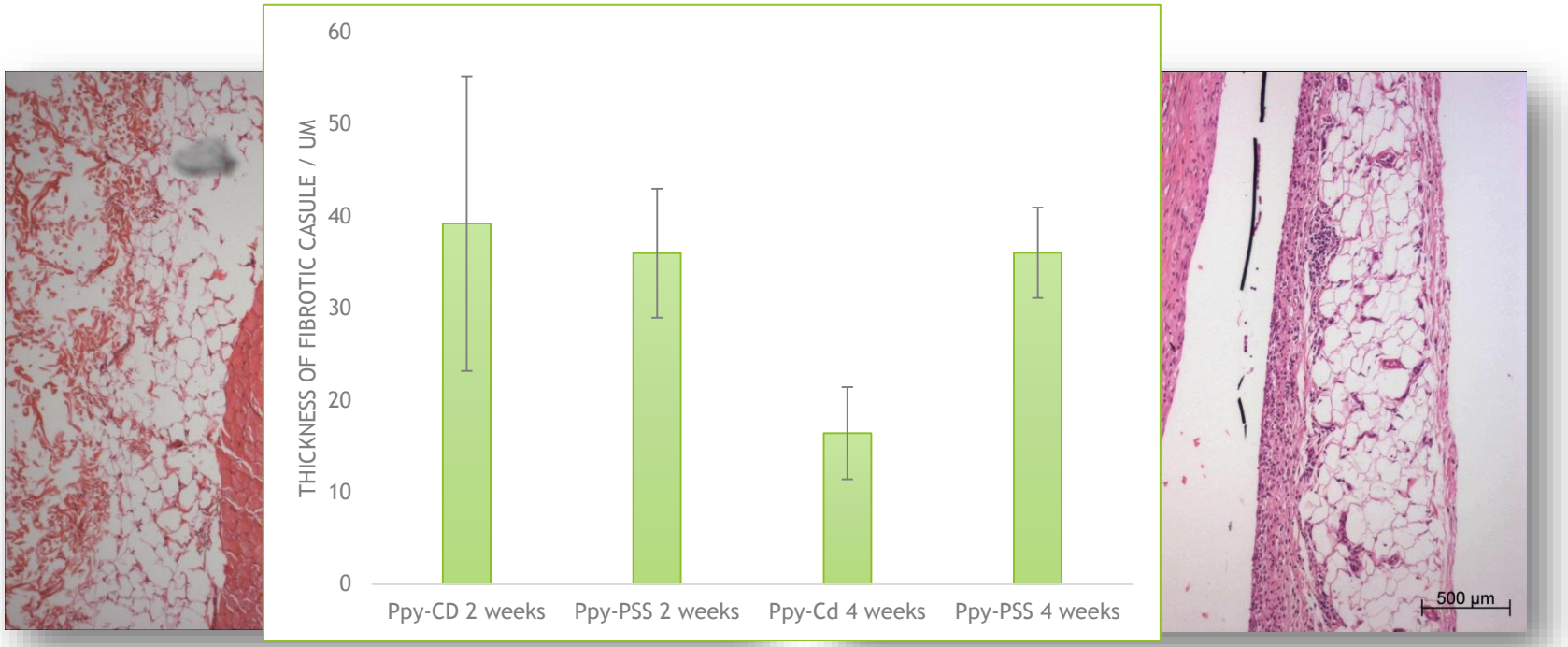


- Fluorescent image of PC-12 cells seeded on Ppy-CD polymer

In vivo assessment - Histology

- Ppy-CD and controls were electrochemically deposited on ITO and peeled off
- They were sterilized using UV light, ethanol and sterile PBS prior to surgery
- 4 Implants (0.5 cm^2) were embedded in the subcutaneous space of each adult Lewis rat and left for 2 and 4 weeks
- A necropsy was preformed
- The tissue samples were prepared:
  - Fixation stabilizes and preserves the tissue.
  - Embedding converts the tissue into a solid form which can be sliced.
  - Sectioning (slicing) provides the very thin specimens needed for microscopy.
  - Staining provides visual contrast and may help identify specific tissue components.
    - Haematoxylin and eosin stain (H&E stain) is one of the most commonly used stains in histology
    - Haematoxylin stains nuclei blue and Eosin stains cyctoplasm and collagen pink/red

In vivo assessment - Histology



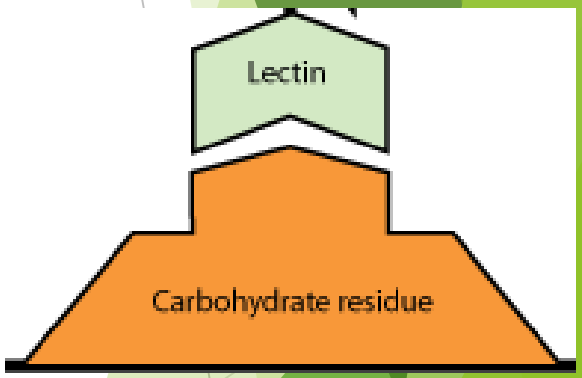
Ppy- CD- 2 weeks

Ppy-PSS – 2 weeks

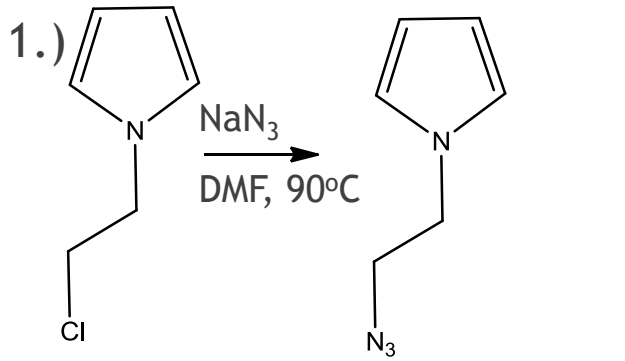
Fibrotic capsule thickness as a function of implant

NUI Maynooth

- Surface modification of a polymer with a chemical function or biomolecules have been shown to strongly influence protein binding and therefore cell material interactions
- Using Carbohydrates because
  - Lectins are specific carbohydrate-binding proteins that recognize and bind to specific carbohydrates found on the surfaces of cells
  - They play a role in interactions and communication between cells typically for recognition
- This sort of interaction has an enormous impact on cell adhesion
- Organic Synthesis and functionalised pyrrole with a carbohydrate
- We have achieved this synthesis based on the Sharpless ‘Click reaction’ which is a azide alkyne Huisgen Cycloaddition reaction in the presence of a copper catalyst

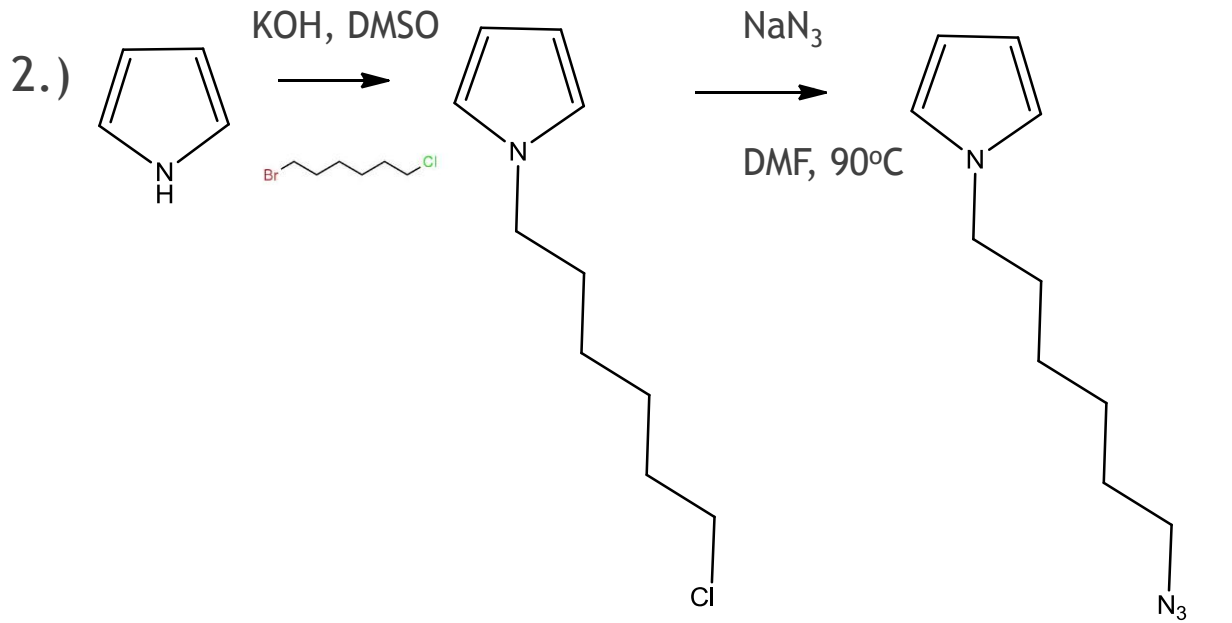


Azido-pyrrole synthesis



1-(2-azidoethyl)-1*H*-pyrrole

92 %



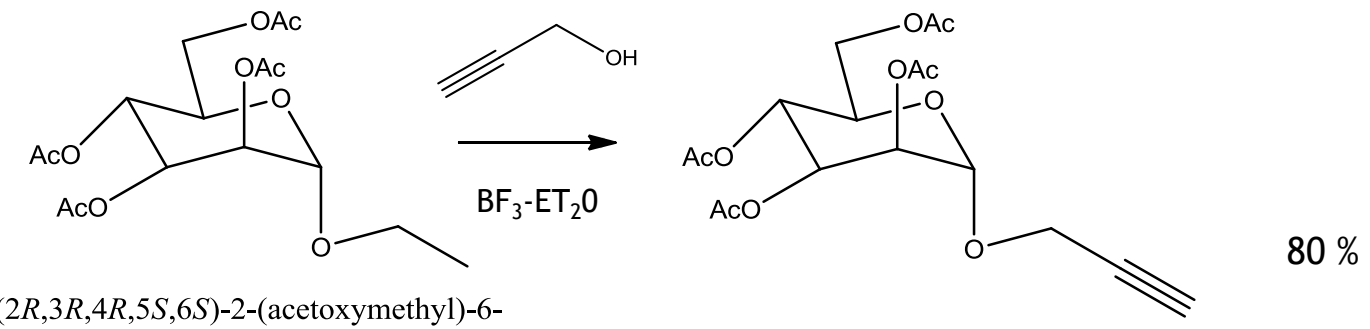
81 %

1-(6-azidohexyl)-1*H*-pyrrole

90 %

Glycosylation reactions

3.)

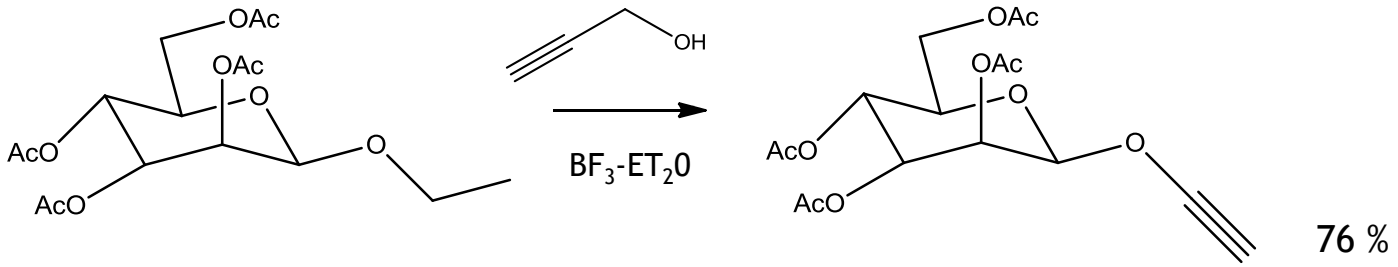


(2*R*,3*R*,4*R*,5*S*,6*S*)-2-(acetoxymethyl)-6-ethoxytetrahydro-2*H*-pyran-3,4,5-triyl triacetate

(2*R*,3*R*,4*R*,5*S*,6*S*)-2-(acetoxymethyl)-6-(prop-2-yn-1-yloxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate

80 %

4.)

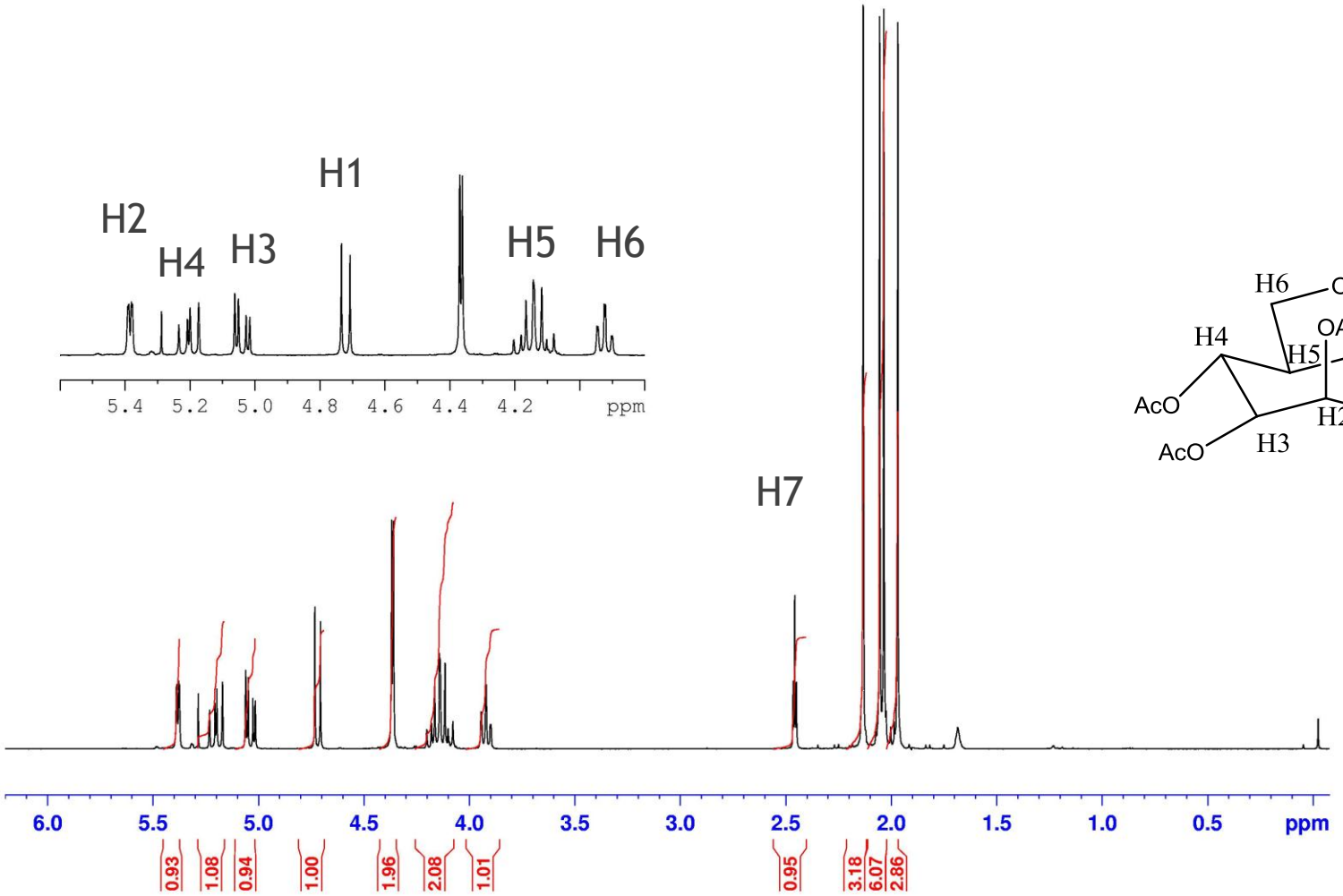


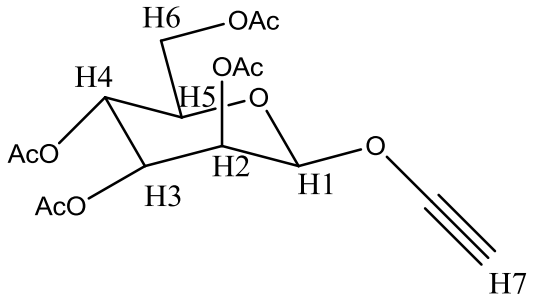
(2*R*,3*R*,4*R*,5*S*,6*R*)-2-(acetoxymethyl)-6-ethoxytetrahydro-2*H*-pyran-3,4,5-triyl triacetate

(2*R*,3*R*,4*R*,5*S*,6*S*)-2-(acetoxymethyl)-6-(ethynyloxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate

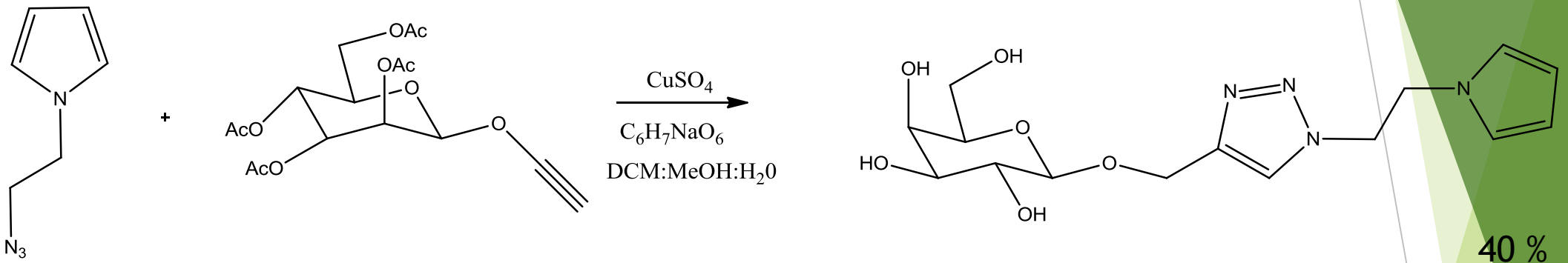
76 %

Galactose Propargyl



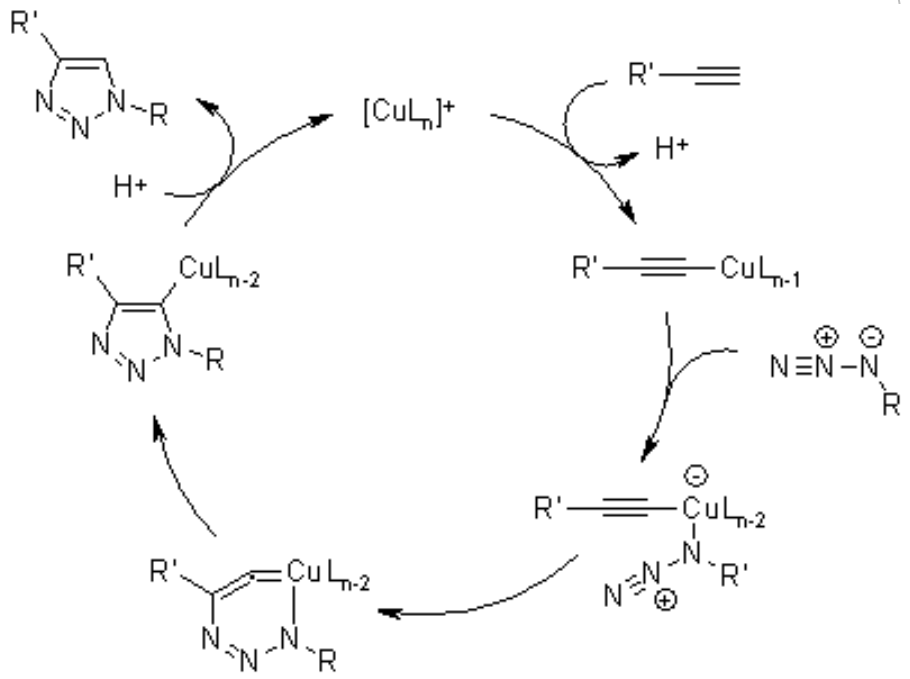


Cyclo-addition reaction products

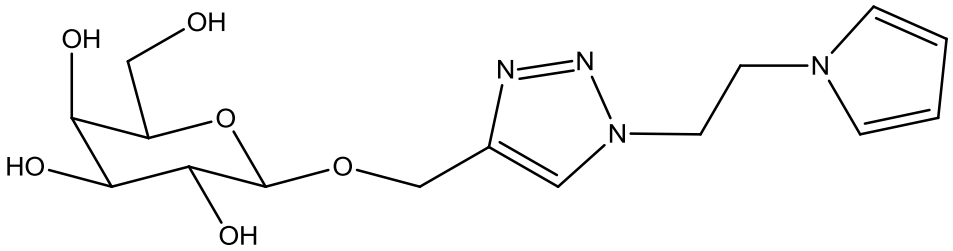


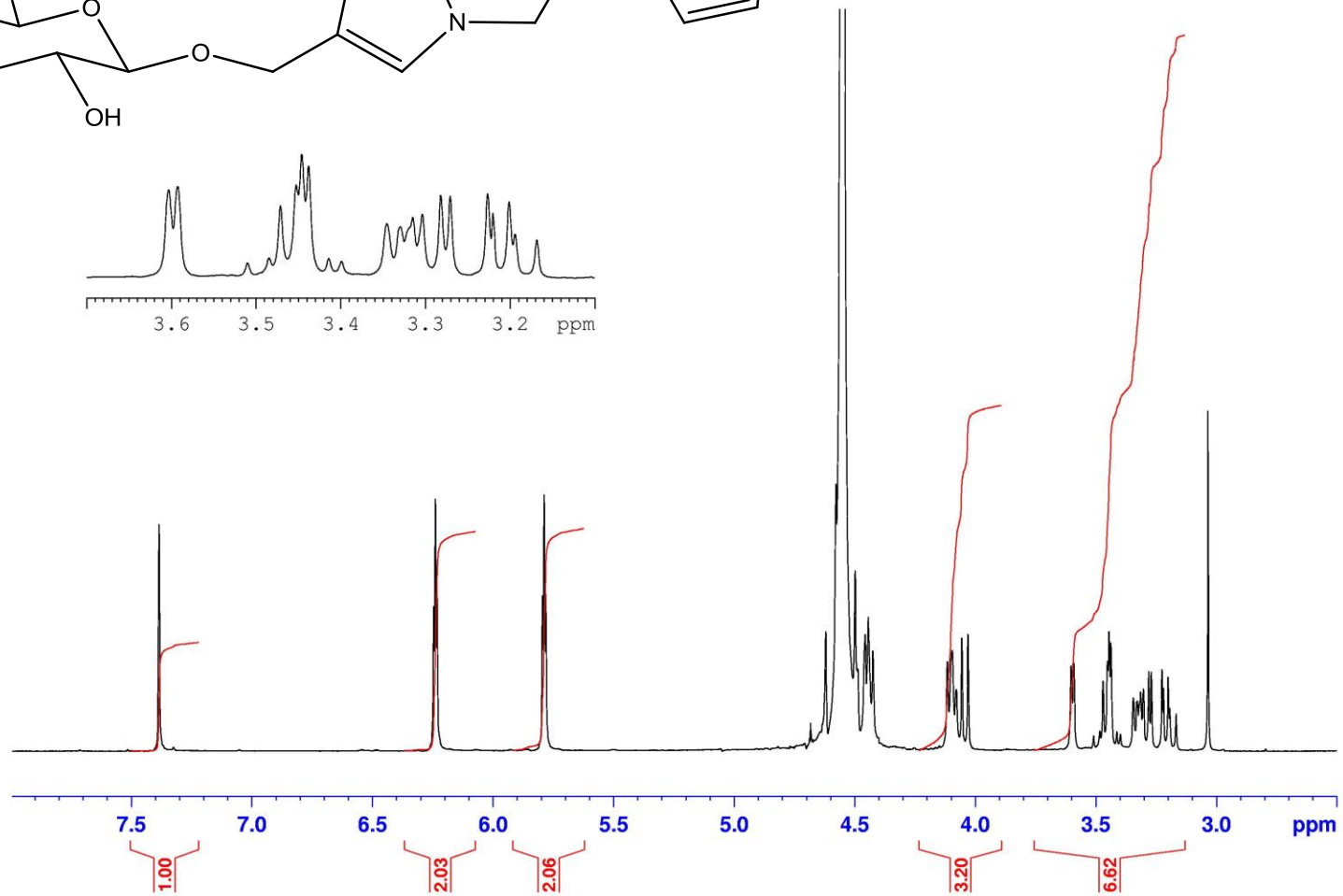
40 %

- The active Cu(I) catalyst is generated
- A copper acetylide forms, after which the azide displaces another ligand and binds to the copper.
- Then, an unusual six-membered copper(III) metallacycle is formed.
- Ring contraction to a triazolyl-copper derivative is followed by protonolysis that delivers the triazole product and closes the catalytic cycle.

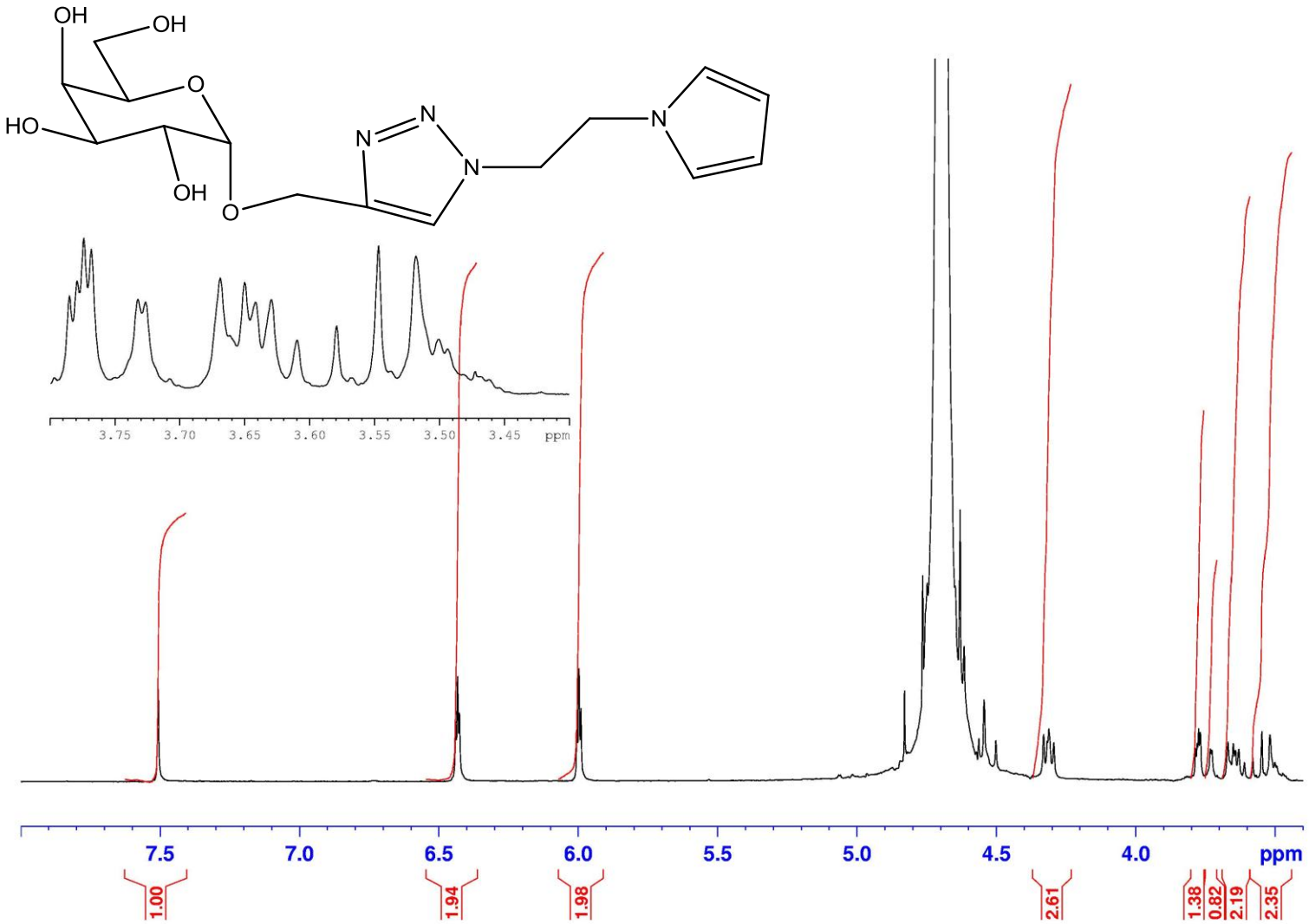


Product Galactosepyrtriazole



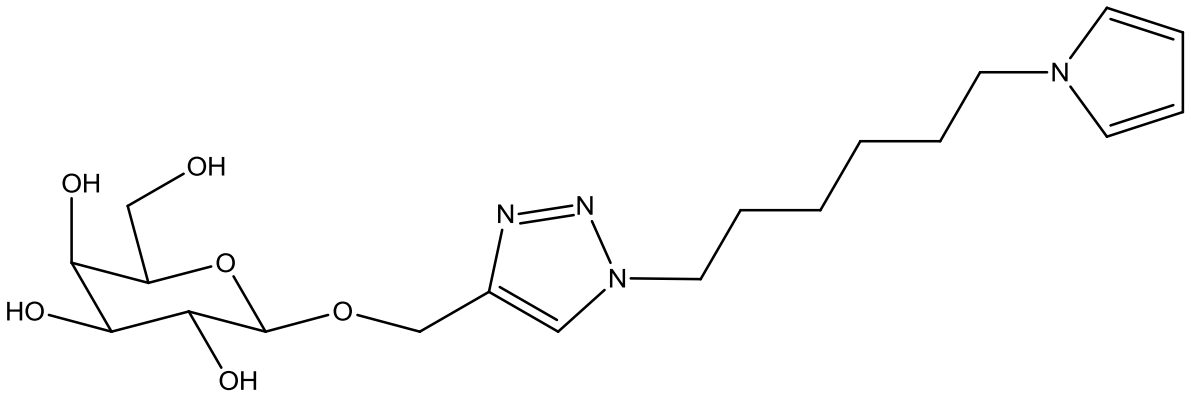


Product Mannosepyrtriazole



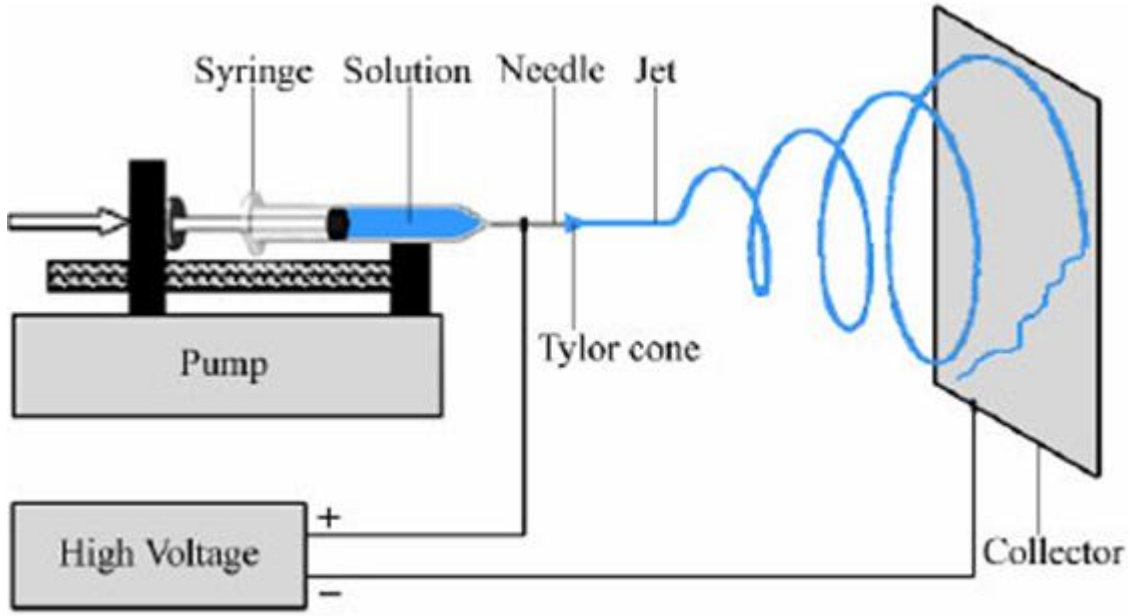
Current step:

- Electrochemical and chemical polymerisation have been unsuccessful with the modified pyrrole to date
  - Steric Hindrance

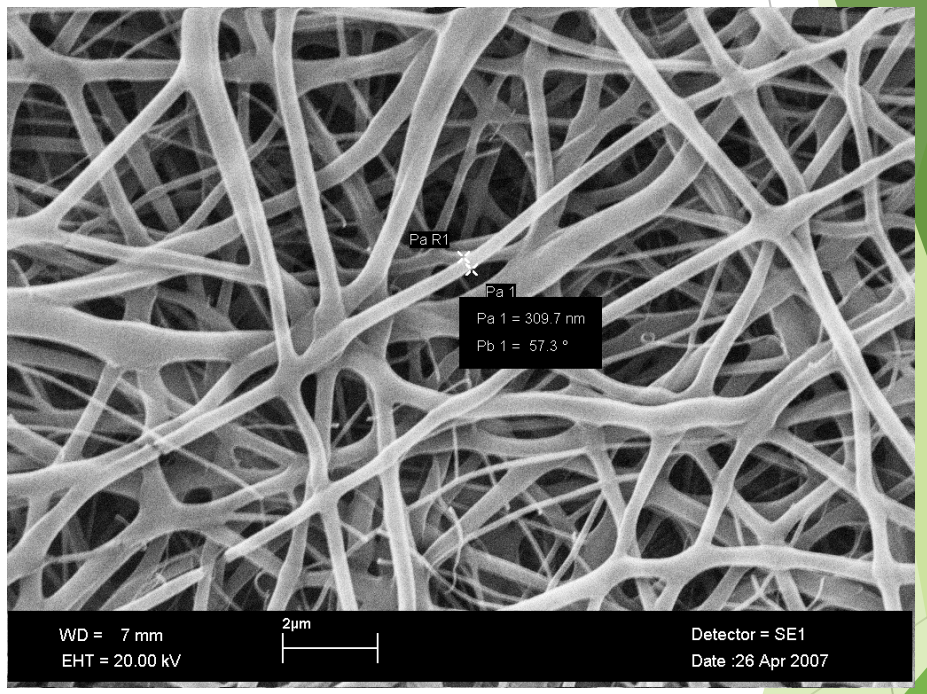


(2*R*,3*R*,4*S*,5*R*,6*R*)-2-((1-(6-(1*H*-pyrrol-1-yl)hexyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol

What is Electrospinning?

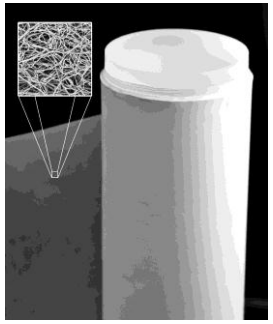


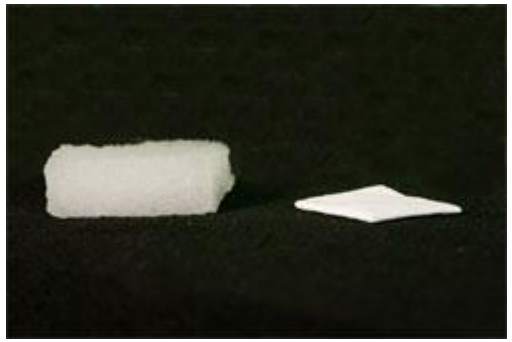
Electrospinning Set-up



SEM of PLGA in HFIP

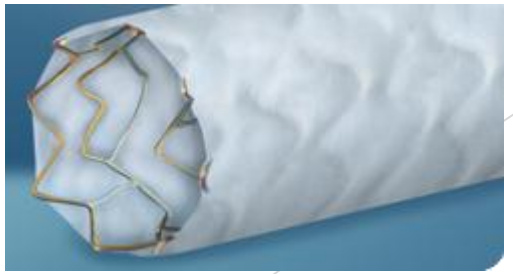
Uses and Products





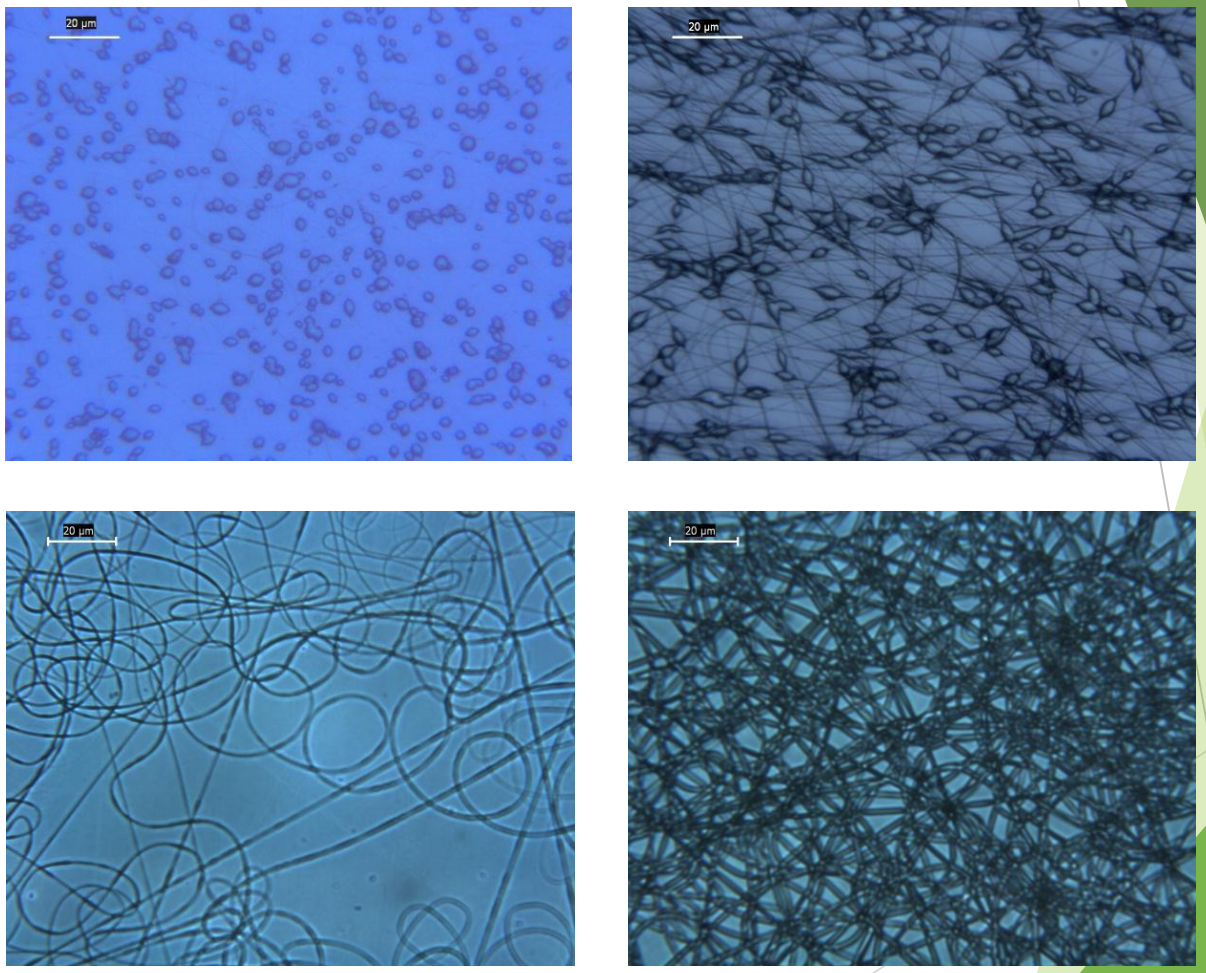
AVflo™ Vascular Access Graft (Center-Coiled Version)

- Filtration
- Textile manufacturing
- **Uses**
- Medical devices
- Composites



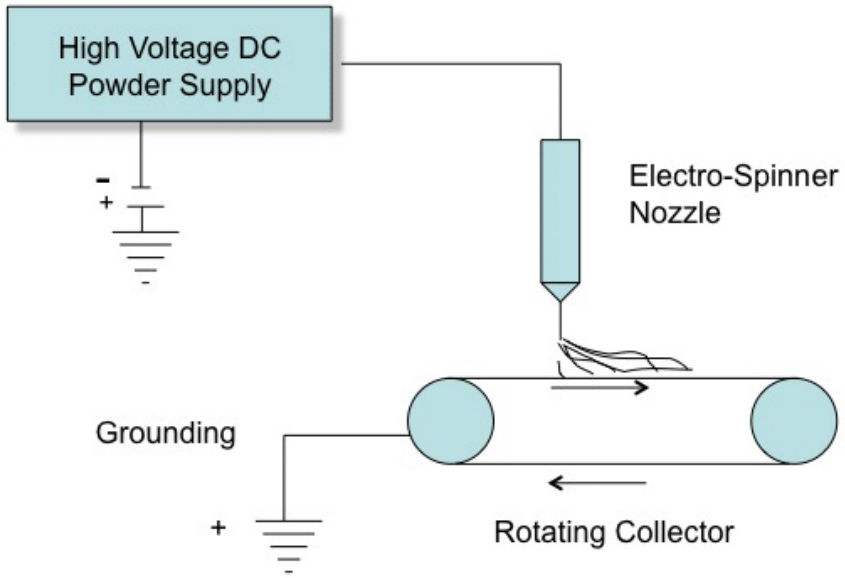
Various Parameters needed to spin

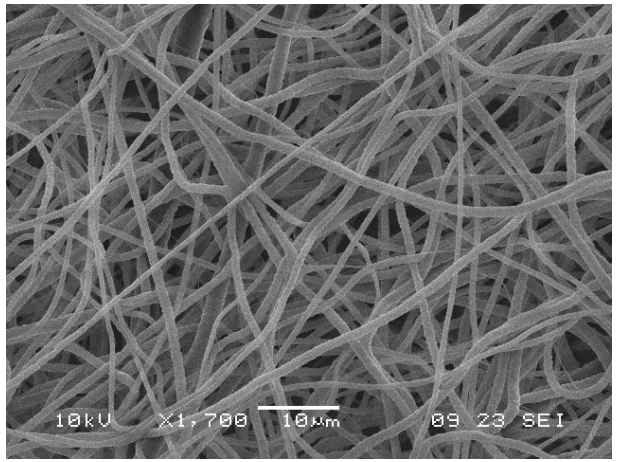
- Process Parameters
  - Voltage
  - Flow rate
  - Concentration
  - Ambient temperatures
  - Distance
  - Motion of substrate
- System Parameters
  - Viscosity
  - Conductivity
  - Molecular weight

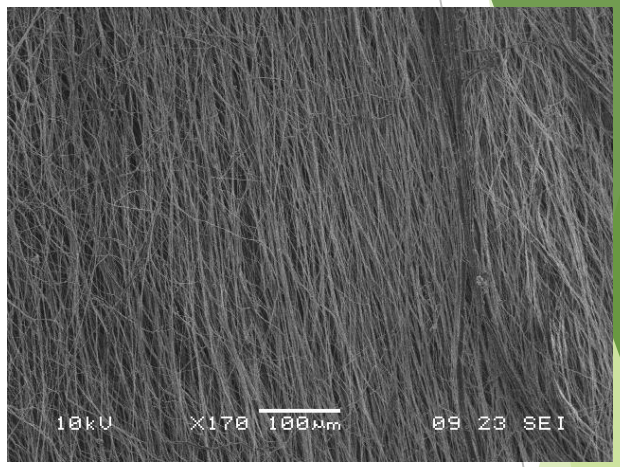


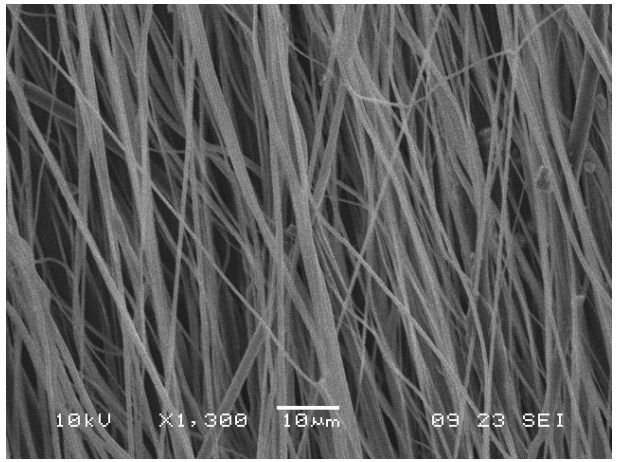
poly(lactic-co-glycolic) acid (PLGA)

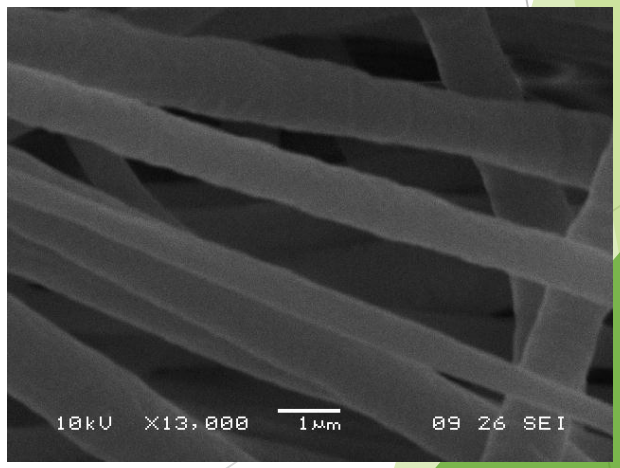
Aligned fibers using a rotating collector









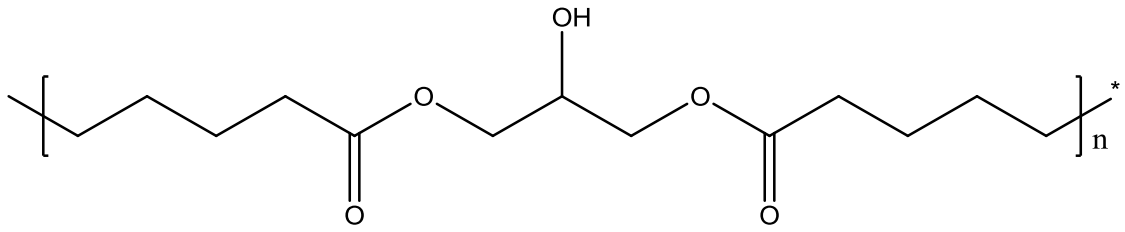


Polyvinyl alcohol (PVA)

Collaboration

Frederick Ghosh, Lund University

- **Retinal degeneration disease affects 30 million patients worldwide**
  - Our collaborators want to make a material for retinal transplantation
- **Poly(glycerol-co-sebacic acid)[9]** (PGS) is a biodegradable elastomer
  - desirable mechanical properties
  - Limitated by difficulties in Casting micro and nanostructures
- Maintain the mechanical characteristics of PGS yet produce nanofibrous structures for potential drug/protein delivery

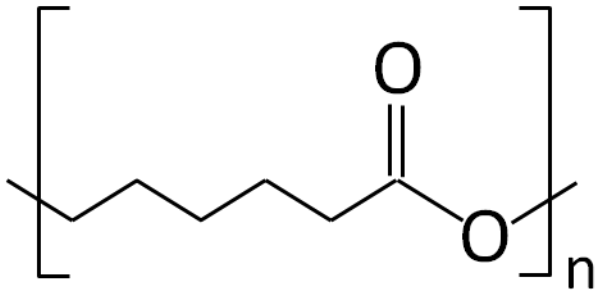


(PGS)

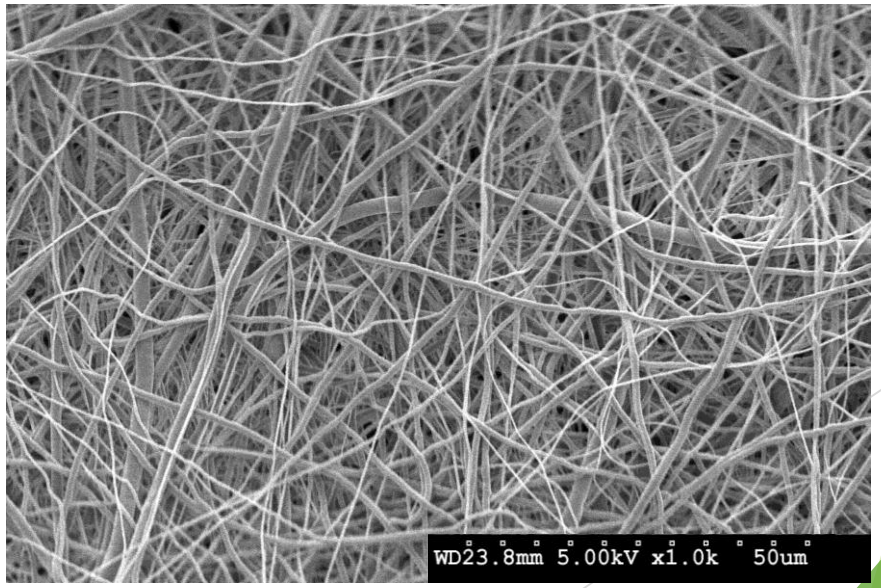
9. Nature Biotechnology 20, 602 - 606 (2002)

Material and Methods

- The PGS disk were cut into 5 × 5 × 3 mm blocks, embedded in Tissue-Tek OCT compound
- Then cryo-sectioned into 30 μm membranes at –30 °C with a heavy duty razor blades
- Slices were then placed in the electrospinning set up and PCl was electrospun onto the fibers at varying thickness



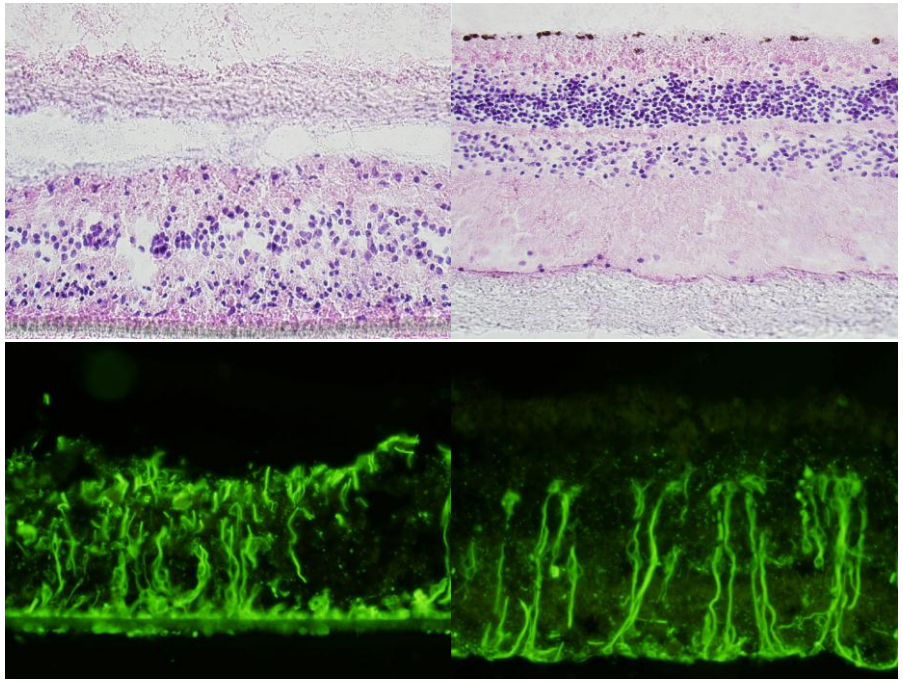
Polycaprolactone (PCL)



SEM of electrospun PCl

Swedish Results

- The membrane adheres well to the retina
- No apparent ingrowth of Müller cell fibers can be seen (vimentin labeling)
- Retinas survive better when cultured with the outer retina up
- Müller cell fibers in the outer retina sprouts through the cultured membrane



Conclusions

- Ppy-CD is a biocompatible polymer
  - Demonstrates good cell adhesion
  - Shows no toxicity
  - *In vivo* shows minimal anti inflammatory response
- ‘Click’ based chemistry is a successful synthesis route for the modification of pyrrole
- Electrospinning is a technique suitable for producing materials for a variety of applications including tissue engineering, wound healing and drug delivery

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