Conducting Organic Materials for Engineering Tissue

Gillian Hendy
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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
Mechanism of Muscle Contraction
Peripheral nerve regeneration

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

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

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.

\[
\begin{align*}
\text{PPy}^0 & \quad \text{Neutral - reduced} \\
\text{Release of Drug} & \quad \text{oxidation} \\
\text{PPy}^+ \ A^- & \quad \text{Reduced state} \\
\text{Anion doping and binding of Drug} & \quad \text{reduction}
\end{align*}
\]
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 its conductivity, biocompatibility and its ability to undergo surface modification with bioactive agents

Materials and Methods

Pyrrole → Polypyrrole

Oxidation Potential

SEM image of Ppy-β-Cyclodextrin

ECIS cell culture plate
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 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.
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) → Mitochondrial reductase → Formazan (purple/Blue)

Journal of Immunological Methods, 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.

<table>
<thead>
<tr>
<th>Structure of Hoechst dyes</th>
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<tbody>
<tr>
<td><strong>Residues</strong></td>
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<tr>
<td>Hoechst 33258</td>
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<tr>
<td>Hoechst 33342</td>
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<tr>
<td>Hoechst 34580</td>
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<td><strong>CAS Number</strong></td>
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</tbody>
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\[ \text{C}_{n} \text{H}_{m} \text{N}_{p} \text{R} \]

\[ \text{FW} = 410.512 + \text{R g/mol} \]
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 cm²) were embedded in the subcutaneous space of each adult Lewis rat and left for 2 and 4 weeks.
- A necropsy was performed.
- 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 cytoplasm and collagen pink/red.
In vivo assessment - Histology

Fibrotic capsule thickness as a function of implant

- Ppy-CD 2 weeks
- Ppy-PSS 2 weeks
- Ppy-Cd 4 weeks
- Ppy-PSS 4 weeks
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.) NaN₃, DMF, 90°C

2.) KOH, DMSO

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

1-(6-azidohexyl)-1H-pyrrole
Glycosylation reactions

3.)

\[
\text{BF}_3\cdot\text{Et}_2\text{O} 
\]

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

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

80%

4.)

\[
\text{BF}_3\cdot\text{Et}_2\text{O} 
\]

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

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

76%
Galactose Propargyl

[Chemical diagram and NMR spectrum showing the labeling of protons H1, H2, H3, H4, H5, H6, and H7.]
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
What is Electrospinning?

Electrospinning Set-up

SEM of PLGA in HFIP
Uses and Products

Filtration

Medical devices

Textile manufacturing

Composites

AVflo™ Vascular Access Graft (Center-Coiled Version)
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

\[\text{PGS} \]\n
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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