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Kidney Tissue Engineering using Polyhydroxyalkanoates

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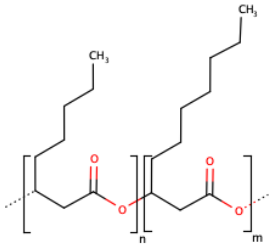
Introduction

Polyhydroxyalkanoates (PHAs) are natural polymers of bacterial origin produced using bacterial fermentation. PHAs are known to be biocompatible towards a broad array of human cells, and have been widely utilised in biomedical applications.[1] A medium-chain-length PHA (mcl-PHA) is selected to develop a bioartificial kidney with the potential of constructing a 'wearable kidney' in the future.[2] Initially, this research will explore glomerular cells for the tissue engineering approach, the conditionally immortalised human podocytes (CIHP).[3] In future, other cell types and additive manufacturing will be combined with the ultimate aim of the development of a mature bioartificial kidney.



mcl-PHA (P(3HO-co-3HD))

Methodology

1 PRODUCTION OF MCL-PHA BY *PSEUDOMONAS PUTIDA*

Fed-batch fermentation

P. putida
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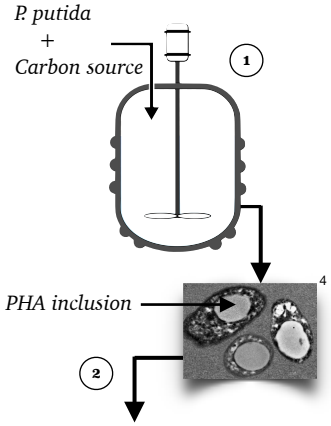
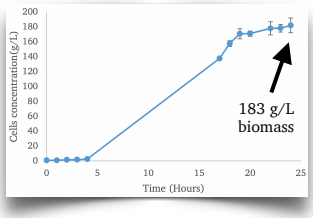
Carbon source

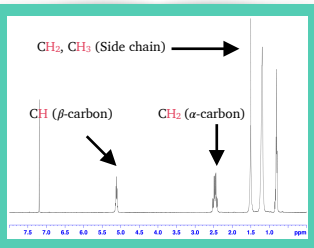


Extraction of the polymer

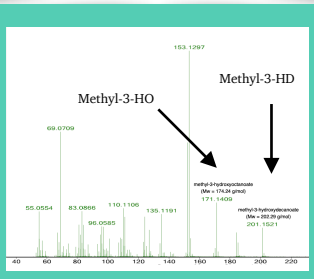
P. putida growth curve in 24 hours



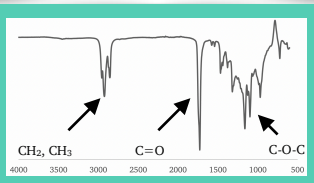
2 CHARACTERISATION OF MCL-PHA



Proton Nuclear Magnetic Resonance ([1]H NMR)

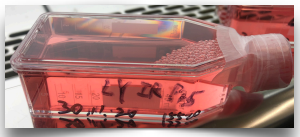


Gas Chromatography-Mass Spectrometry (GC-MS)

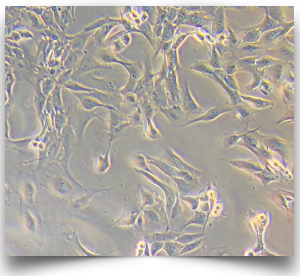


Fourier Transform Infrared Spectroscopy (FTIR)

3 GROWTH OF CIHP CELL LINE



Culture in flask



CIHP under microscope

Cells grown on mcl-PHA

Assay for viability
Resazurin

Antibody assays
Nephrin
Podocin
Synaptopodin

Summary

Fermentation was done in 30L fed-batch fermenter, utilising *Pseudomonas putida*. The mcl-PHA inclusion was extracted and solvent cast into polymer films.

The mcl-PHA was characterised using: NMR to determine structure; GC-MS to determine monomer composition; and FTIR to determine functional groups

CIHP cells is grown onto the mcl-PHA and assayed for viability in terms of growth and physiological performance.

The polymer will be potentially 3D printed in to a bioartificial kidney construct with CIHP.

References

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2. Tarantal, A.F. and C.A. Batchelder, *Scaffolds for kidney tissue engineering.* Handbook of Tissue Engineering Scaffolds, Vol 2, 2019: p. 477-492.
3. Kitching, A.R. and H.L. Hutton, *The Players: Cells Involved in Glomerular Disease.* Clinical Journal of the American Society of Nephrology, 2016. 11(9): p. 1664-1674.
4. Gumel, A.M., Annuar, M.S.M., & Heidelberg, T.. (2014). Growth kinetics, effect of carbon substrate in biosynthesis of mcl-PHA by Pseudomonas putida Bet001. *Brazilian Journal of Microbiology*, *45*(2), 427-438