Cardiovascular Tissue Engineering Using Natural Polymers

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Introduction

- Cardiovascular diseases such as **myocardial infarction (MI)** are the leading cause of death globally.
- Due to the **poor regenerative capacity** of the heart, current treatments are able to mitigate MI symptoms but are unable to repair the damaged tissue.
- Polyhydroxyalkanoates (**PHAs**) are **natural polymers** produced by **bacteria**.

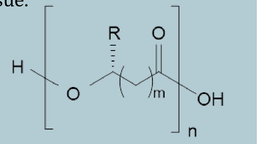

Figure 1. General structure of PHAs

- PHAs are **FDA approved** biopolymers that have been shown to be **biocompatible** and **bioresorbable**.
- They can **degrade** by surface erosion to produce **non-immunogenic** products.
- Medium chain length PHAs such as **P(3HO-*co*-3HD)**, have **elastomeric** properties, making them ideal for cardiac applications

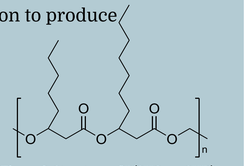

Figure 2. Structure of P(3HO-*co*-3HD)

- Due to their **flexibility**, PHAs' surface properties can be modified and they are amenable to complex fabrication techniques including **3D printing** to produce highly vascularised constructs that can provide the required biomimetic environment for **cell adhesion, growth and proliferation**.
- **Alginate** is a natural polymer that can form a **hydrogel**, making it an ideal material for cell encapsulation

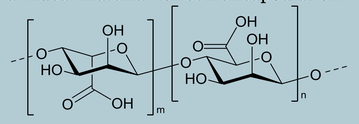

Figure 3. Structure of alginate

Production and extraction of PHAs

- Bacteria is cultured in a bioreactor with a carbon source and under nitrogen limiting conditions
- PHA is stored intracellularly
- PHA is purified and extracted via the process of Soxhlet extraction

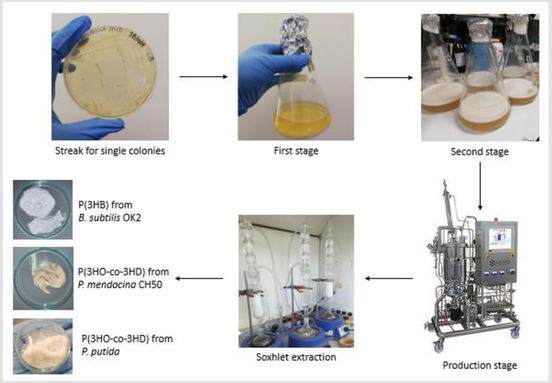

Figure 4. Step-wise production of PHAs

3D multimaterial printing of PHAs and alginate

- Woodpile designs with alternating logs of P(3HO-*co*-3HD) and alginate were 3D printed

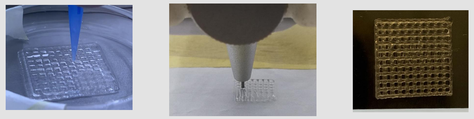

Figure 5. 3D printing of P(3HO-*co*-3HD) and alginate

Cell cytotoxicity assay results

- C2C12 mouse-derived myoblast cells were used for initial cytotoxicity assays of the P(3HO-*co*-3HD) and alginate
- 3 different crosslinking solutions for preparing the alginate were compared

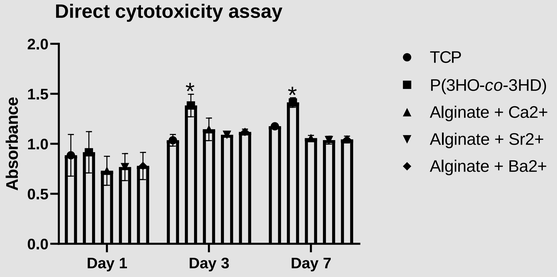

Figure 5. Cells were seeded directly onto the polymer samples. The results show that P(3HO-*co*-3HD) significantly improves cell survival from 3 days after seeding, and all alginate samples were not significantly different to tissue culture plastic (TCP). Two-way ANOVA, multiple comparisons, all samples compared against TCP as control, N=3, n=3

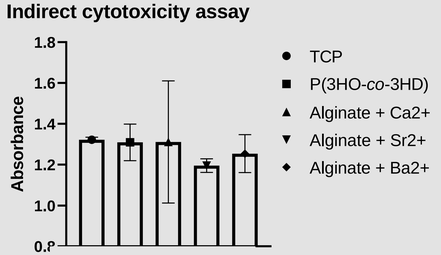

Figure 6. Cells were incubated in media with eluents from the polymer samples . The results show that P(3HO-*co*-3HD) and all alginate samples were not significantly different to tissue culture plastic (TCP). One-way ANOVA, multiple comparisons, all samples compared against TCP as control, N=3, n=3

Conclusions and future work

- P(3HO-*co*-3HD) has been successfully made, purified, and extracted.
- Multi-material patches have been 3D printed
- Trial experiments 3D bioprinting C2C12 cells encapsulated in alginate have been initiated
- Human induced pluripotent stem cells (hiPSCs) have been grown and differentiated into cardiomyocytes (hiPSC-CMs), with initial testing of seeding onto P(3HO-*co*-3HD) compared to fibronectin:

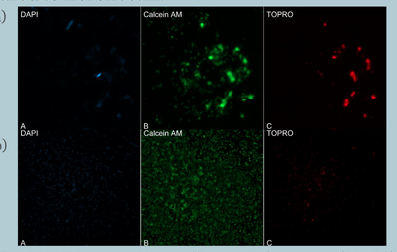

Figure 7. hiPSC-CMs stained for live (Calcein AM) and dead (TOPRO) cells on a) fibronectin, and b) P(3HO-*co*-3HD)

References

1. Rai R, Yunos DM, Boccaccini AR, Knowles JC, Barker IA, Howdle SM, Tredwell GD, Keshavarz T, Roy I. Poly-3-hydroxyoctanoate P(3HO), a Medium Chain Length Polyhydroxyalkanoate Homopolymer from Pseudomonas mendocina. Biomacromolecules 12, 2126, 2011
2. Bagdadi AV, Safari M, Dubey P, Basnett P, Sofokleous P, Humphrey E, Locke I, Edirisinghe M, Terracciano C, Boccaccini AR, Knowles JC, Harding SE, Roy I. Poly(3-hydroxyoctanoate), a promising new material for cardiac tissue engineering. Journal of Tissue Engineering and Regenerative Medicine 12, 495, 2018

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