Developing a mcl-PHA based 3D *in vitro* platform for improved pulmonary cancer modelling

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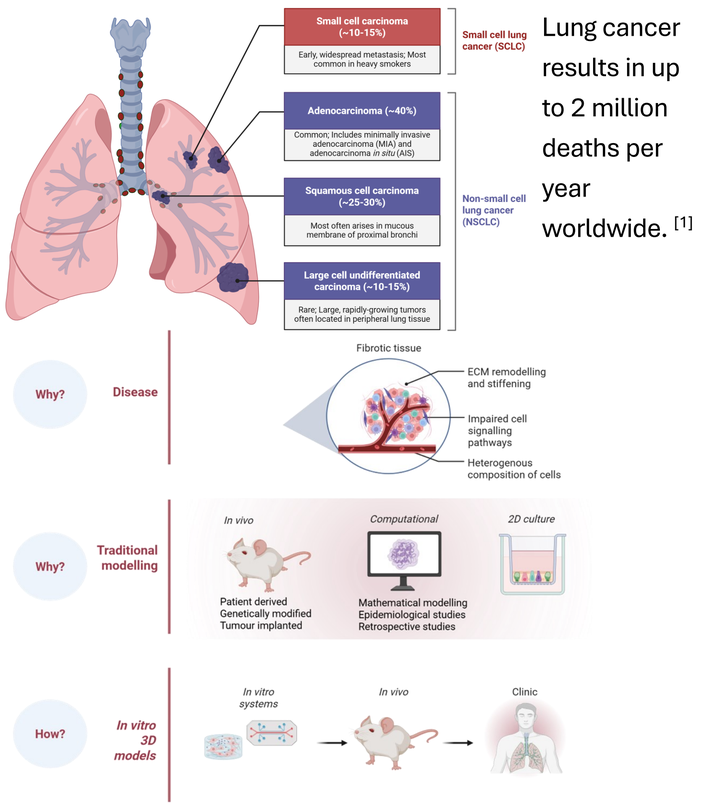
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Background



Lung cancer results in up to 2 million deaths per year worldwide. [1]

Traditional models offer a limited view into the complexity and heterogeneity of the tumour microenvironment leading to poor translational relevance. [2]

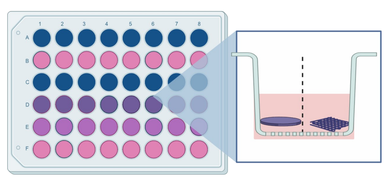
Project aims

To develop a reproducible 3D *in vitro* lung cancer model under dynamic perfusion.

- Initial cytocompatibility of medium chain length polyhydroxyalkanoate (mcl-PHA) with lung cells
- Reproducible 3D printed mcl PHA scaffolds
- Optimised conditions for healthy and cancer cell growth
- Phenotypic analysis of cells under different culture conditions .

Platform design

Static model



Experimental arm: mcl PHA solvent cast film (2D model) or mcl PHA 3D printed scaffolds (3D model)

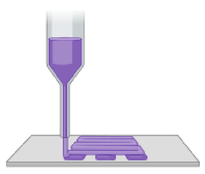
Controls: TCP and collagen coated wells

Timepoints: Days 1,3,7 and 10

Scaffold fabrication



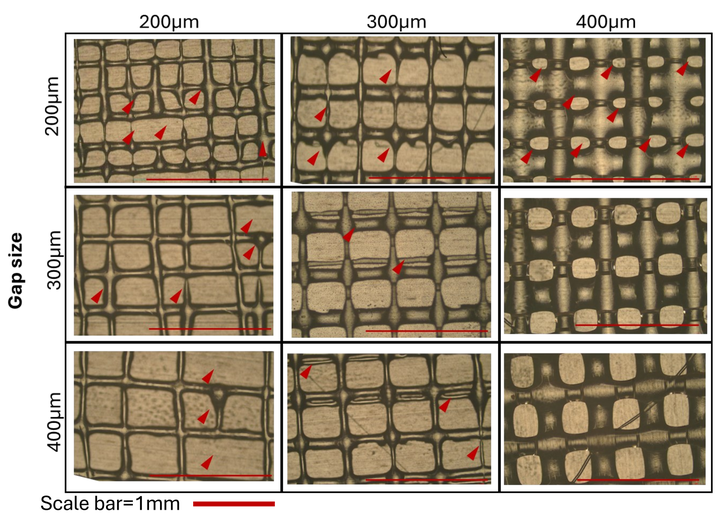
Polymer production using a fed-batch bioreactor set up under nutrient limitation



Printing woodpiles using an optimised melt extrusion 3D printing workflow

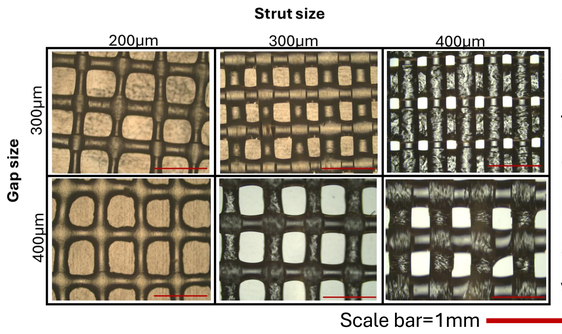


Store in a sealed petri dish and sterilise when needed



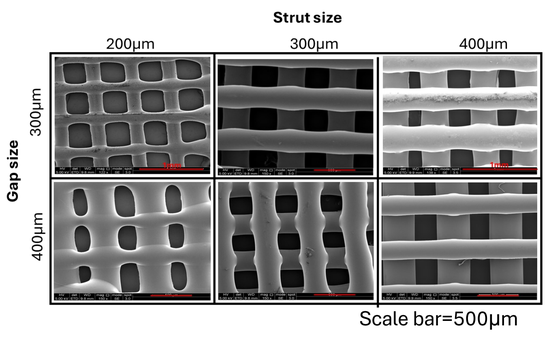
Scaffold morphology



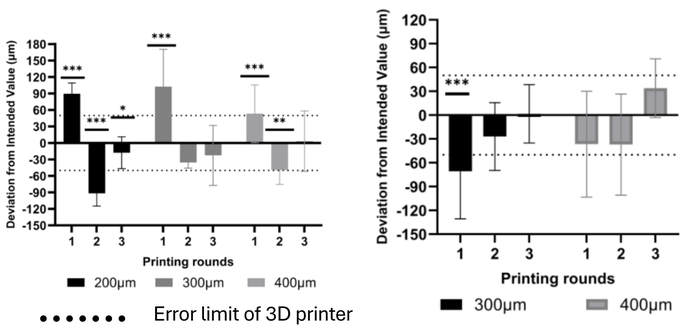


Optimisations to printing conditions

Reduced appearance of visual defects.

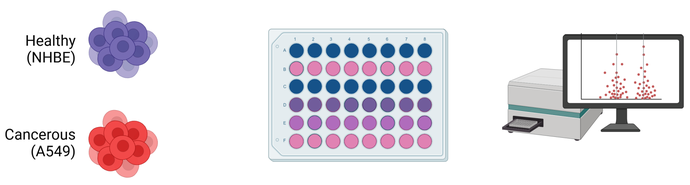


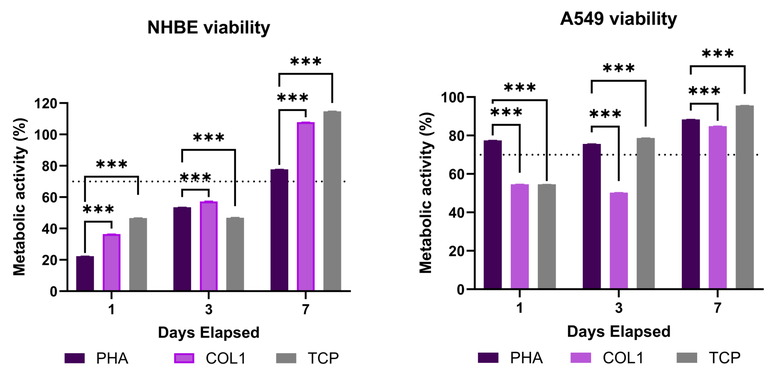
SEM imaging confirms no topographical defects that could impact cell behaviour



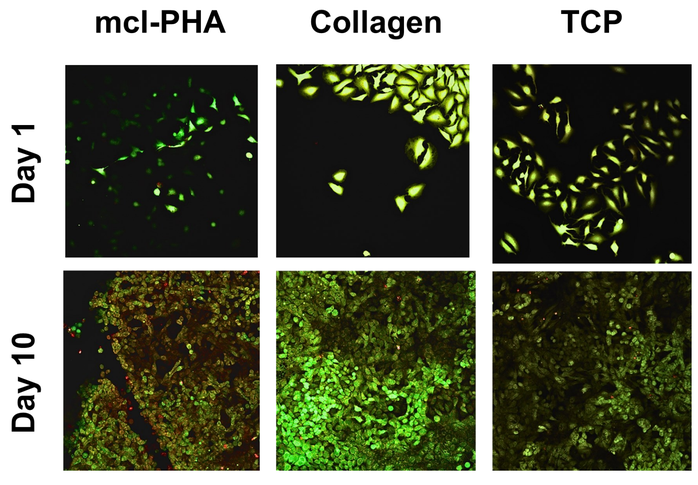
With successive printing rounds, deviation of prints reduced, resulting in reproducible constructs

Cytocompatibility



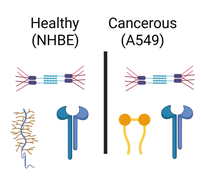


Live/Dead staining– A549 cells

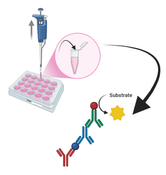


- **Viability:** >70% in all groups by day 7 → differences reflect **functional changes, not cytotoxicity**
- All substrates show significant growth across the two time points
  - Mcl-PHA (both 2D and 3D) show fewer dead cells by day 10
  - Collagen and TCP have a greater proportion of dead cells due to overcrowding

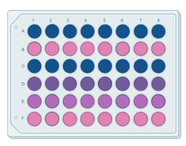
Pulmonary functionality



ELISA panel design dependent on cell type

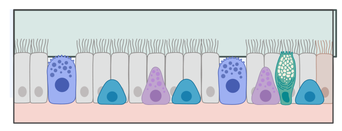


A549 and NHBE ELISA sample collection over 10 days

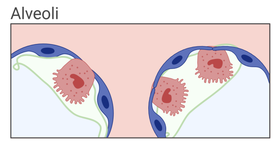


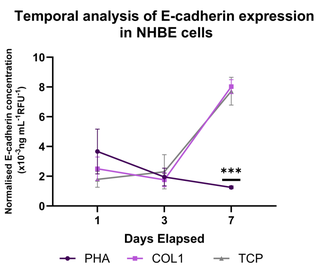
Concurrent resazurin assay for normalisation

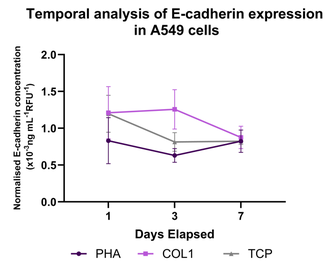
Bronchioles to alveoli



Alveoli

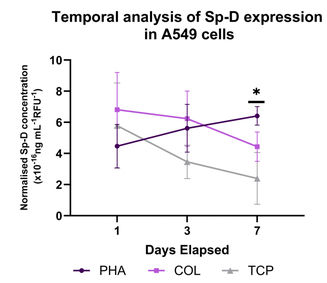






E-cadherin: mcl-PHA promotes **reduced shedding / altered adhesion dynamics** in healthy cells[3]; cancer cells remain **low**, consistent with epithelial–mesenchymal transition-like behaviour. [4]

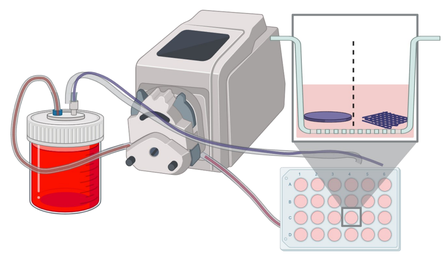
Surfactant**:** Experimental group shows **modest increase in functional activity**



Future work

Carry out Immunofluorescence to observe localisation of markers integral to lung functionality

Dynamic perfusion model



Experimental arms:

1.Static vs Dynamic culturing

2. 2D vs 3D model of mcl PHA

Controls: TCP and collagen coated wells

Timepoints: Day 1,3,7 and 10

Conclusions

- Mcl-PHA solvent cast films allowed for >70% viability by day 7 of a direct resazurin; is biocompatible for both healthy and cancerous lung cells.
- A melt-extrusion 3D printing workflow was sufficiently optimised to produce reproducible woodpile constructs using mcl-PHA within ±50µm error limit
- Time course phenotypic profiles show cell specific patterns for E-cadherin and Sp-D– this potentially demonstrates a more mature profile when cultured on mcl-PHA.

References

[1]J. Zhou, Y. Xu, J. Liu, L. Feng, J. Yu, and D. Chen, "Global burden of lung cancer in 2022 and projections to 2050: Incidence and mortality estimates from GLOBOCAN," *Cancer Epidemiology*, vol. 93, no. 102693, p. 102693, Nov. 2024, doi: https://doi.org/10.1016/j.canep.2024.102693.

[2]H. Joo, S. Min, and S.-W. Cho, "Advanced lung organoids for respiratory system and pulmonary disease modeling," *Journal of tissue engineering*, vol. 15, Jan. 2024, doi: https://doi.org/10.1177/20417314241232502.

[3]S.-Y. Sun, X. Chen, B. Li, and X.-Q. Feng, "Adhesion-regulated dynamics of cells," *Biophysical Journal*, vol. 124, no. 19, pp. 3124–3138, Oct. 2025, doi: https://doi.org/10.1016/j.bpj.2025.01.014.

[4]J. Sun *et al.*, "Metabolic landscape of human alveolar type II epithelial cells undergoing epithelial-mesenchymal transition induced directly by silica exposure," *Journal of Environmental Sciences/Journal of environmental sciences*, Mar. 2024, doi: https://doi.org/10.1016/j.jes.2024.02.020.

Conflict of Interest: IR is a director of PHAst Ltd (CSO) that develops biomedical grade PHAs for a variety of applications including healthcare and biomedical applications. However the mcl-PHA used for this work was produced in the Roy Lab.

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