Beefing Up Cultivated Meat Production With Mycelial Microcarriers

Alice Johnson[1], Amélie Savers[2], Sandesh Meyler[3], Paul Topham[1], Mariana Petronela Hanga[4], Jean-Baptiste Souppez[1], Eirini Theodosiou[1]

[1]School of Engineering and Innovation, Aston University, Birmingham, UK
[2]Myconeos Ltd., BioCity, Nottingham, UK
[3]Multus Biotechnology, London, UK
[4]Department of Biochemical Engineering, University College London, UK

Add me on LinkedIn!

ajohn23@aston.ac.uk

1 Meat-ing the demand

9.8 billion by 2050(1)

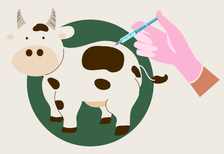
And with it, **demand for meat** is set to increase to **455 million tonnes**(2)

Cultivated meat has the **potential to replace 1 billion cows**(3) with only 100 donor cows (maybe even just 1)(4)

However, efficient ***expansion*** and ***differentiation*** of adherent cells (stages 2 and 3 of the bioprocess) are key challenges(5)

Edible microcarriers (MCs) offer a scalable, cost-effective solution for cell growth and can improve **nutritional** and **sensory** qualities of cultivated meat(6)

2 The cultivated meat bioprocess: from biopsy to bowl

1 

BIOPSY
Stem cells are isolated

2 

PROLIFERATED
Fed with oxygen and nutrients to encourage expansion

3 

DIFFERENTIATED
Stimulated to turn into meat cells (e.g. muscle and fat)

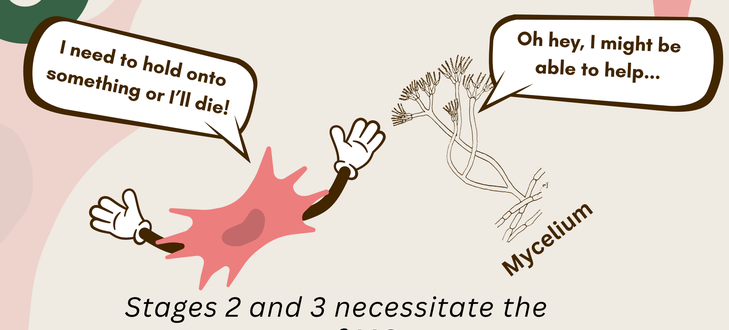
4 

ASSEMBLED
3D-printed/ layered/ aggregated

5 

MANUFACTURED
Processed into products or sold as whole cuts

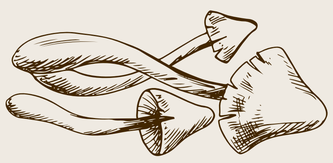
3 Aims & Objectives



Stages 2 and 3 necessitate the use of MCs

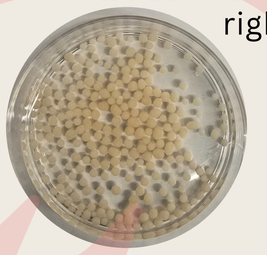
1. Develop **scalable**, **edible** and **cost-effective** MCs that support cell attachment and proliferation in large-scale bioreactors
2. Assess **mycelia (Myc)**, provided by Myconeos, in terms of their effectiveness for scaling adherent cell cultures (starting with bovine mesenchymal stem cells; bMSCs)
3. Test the performance of Myc MCs using industry-relevant, **animal-free media** formulations, provided by Multus, to replace foetal bovine serum (FBS)
4. Evaluate the potential of Myc MCs to produce a **desirable end product** that will be **accepted** by consumers

4 The development of mycelial microcarriers

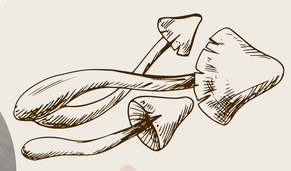


Myc are cultivated from spores in liquid broth (left)

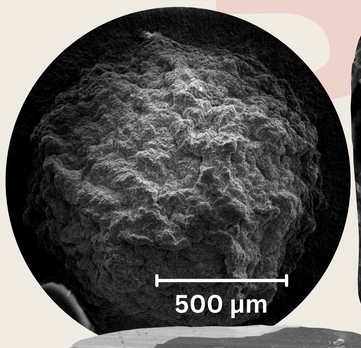
Some naturally form spheres (bottom left), known as pellets, whilst others clump (bottom right)

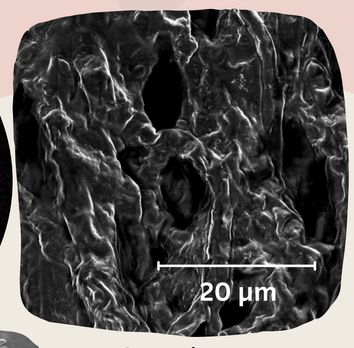


Initial visual screening was used to select strains that formed pellets and subsequently exhibited high surface area

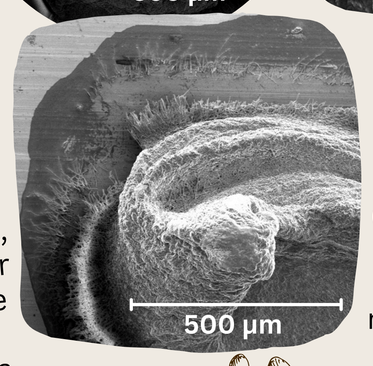


The Myc strains selected range from 50 µm to 2 mm in size





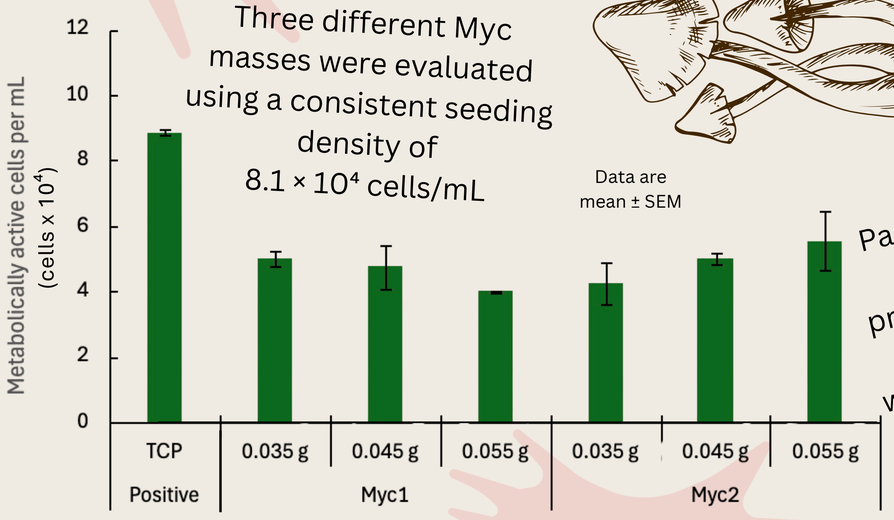
A 60× image (right) captures the edge of a dehydrated Myc pellet, revealing its distinctive, high surface area, 'hairy' outer layer formed by dense networks of hyphal filaments



SEM images highlight the fibrous, porous structure of Myc pellets, shown at 65× magnification (top left) and 2000× magnification (top right)

Two of the four strains were randomly selected for cell attachment studies

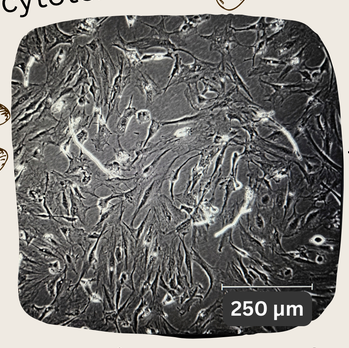
Three different Myc masses were evaluated using a consistent seeding density of 8.1×10^4 cells/mL



Factors such as spore concentration, mixing, temperature and growth duration determine their size, colour and morphology (below)

Parameters were optimised to produce relatively uniform pellets with a high surface area, whilst maintaining ease of handling

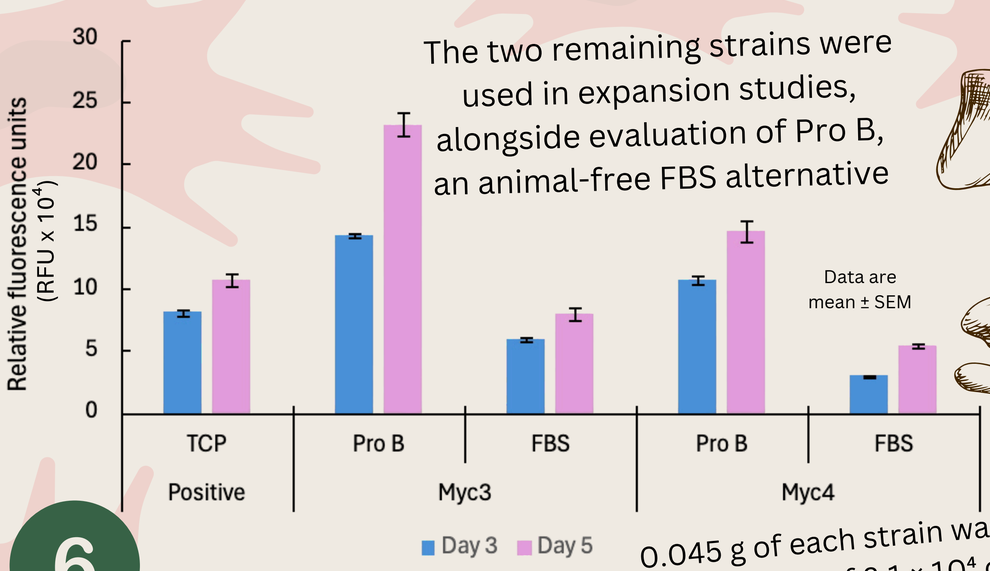
bMSCs were seeded in well plates containing autoclaved Myc in cell strainers to test for potential cytotoxicity

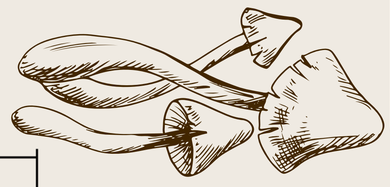


Persistent presence of hyphal fragments and spores were noted in wells (left), even after two DPBS washes—possibly interacting with bMSCs

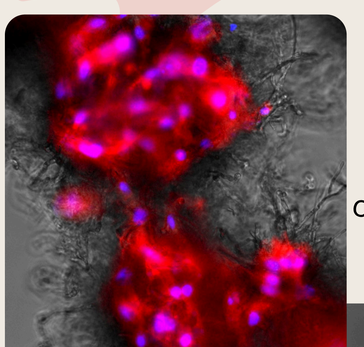
Numerous strains supported cell growth with typical stem cell morphology. Four of the most promising strains were selected for further experiments

The two remaining strains were used in expansion studies, alongside evaluation of Pro B, an animal-free FBS alternative

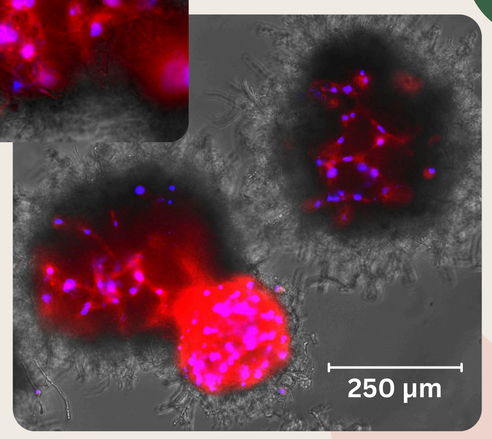




0.045 g of each strain was seeded at a density of 8.1×10^4 cells/mL



Hoechst and phalloidin staining indicates extensive stem cell growth on the Myc3 MCs



5 Future Work

Future experiments will focus on optimising cell culture conditions (e.g. microcarrier seeding density, microcarrier size, etc.) as well as fully characterising the best strains and evaluating their behaviour in spinner flasks

6 References

1. UN (2017) https://www.un.org/en/desa/world-population-projected-reach-98-billion-2050-and-112-billion-2100; 2. Our World in Data (2012) https://ourworldindata.org/grapher/global-meat-projections-to-2050; 3. Ritchie et al. (2023b) https://www.statista.com/statistics/263979/global-cattle-population-since-1990/; 4. Melzener et al. (2020) doi: 10.1002/jsfa.10663; 5. Bellani et al. (2020) doi: 10.3389/fnut.2020.575146; 6. Yen et al. (2023) doi: 10.1038/s41467-023-38593-4