Designing the future of cultivated meat: An Uncommon journey to cost-effective stem cell growth media and RNA delivery

Jonathan Faherty[1], Alex Rimmer[2], Samuel East[2], Catriona Jamieson[2]
[1]SPT Labtech, Melbourn, Cambridgeshire, UK [2]Uncommon, Cambridge, Cambridgeshire, UK

Introduction

Cellular agriculture could be instrumental to environmentally-friendly food production. However, high raw material costs impede financial viability. One area that could be improved is stem cell culture media, which are often expensive and can contain components unsuitable for food production. Uncommon, a UK-based biotechnology company, has sought to develop low-cost, animal-free culture media to produce cultivated meat as an alternative to traditionally farmed meat (Figure 1).

This poster highlights how the Uncommon team have applied automated liquid handling and Design of Experiments (DoE) methodology to drive the development of low-cost, animal-free media, and optimize cost-effective RNA delivery.

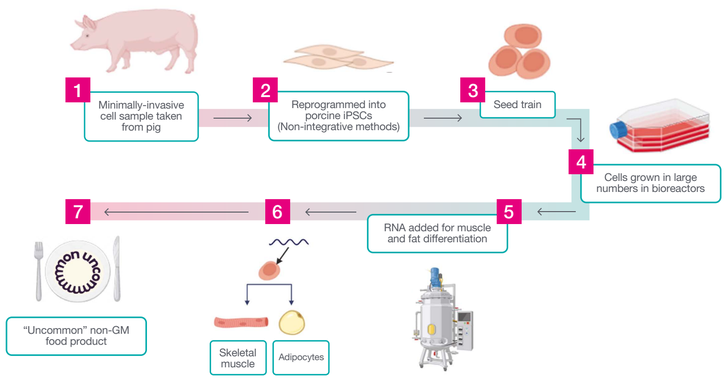


Figure 1. Uncommon's process to produce cultivated meat at scale. Considerations need to be taken at every step to reduce the cost impact of the final product.

Development of 2D piPSC growth media

- The team investigated the impact of four media growth factor supplements (A, B, C and D) at three different concentrations in parallel. JMP and Synthace software generates unique trial formulations (Figure 3) and provides a set of instructions for the dragonfly discovery liquid automated dispenser to create media experiments
- 2D cultures in 384-well plates are inoculated with 300 µL media, incubated, and feed stocks are replenished using apricot S3.
- Cultures are stained with DAPI and imaged. Cell count/well is measured and fed back into JMP to further optimize growth media.

Figure 2. Iterative process used to optimize growth media for 2D iPSC cultures.

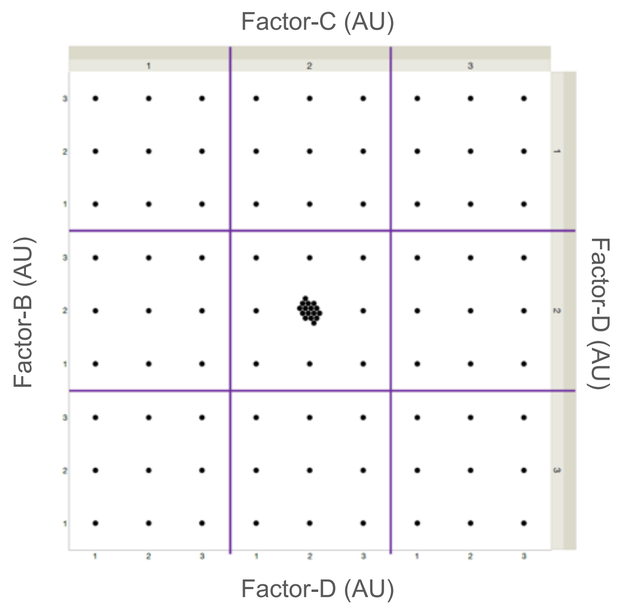


Figure 3. Design space visualization of the conditions that were tested in 2D cell culture. Replicates of the central formulation were included to investigate inherent biological variation.

Initial data showed that Factor-A strongly promoted 2D cell growth, while Factor-D impeded 2D cell growth (Figure 4). To investigate further, the workflow was repeated using a broader range of Factor-D concentrations

The resulting data (Figure 5) confirmed that Growth Factor-D is necessary for cell growth, but exhibits a critical threshold, after which further supplementation impedes cell growth. This result was consistent across rHuman and rPorcine homologs of Factor D, showing the team's ability to rapidly formulate optimized iPSC growth media and respond proactively to a shifting regulatory landscape.

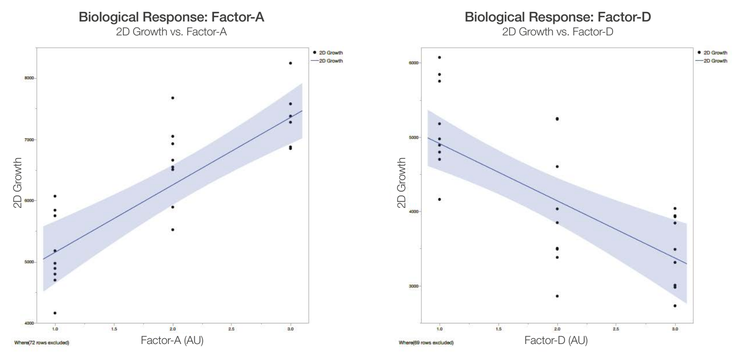


Figure 4. Initial data showed that Factor-A strongly promoted 2D cell growth, while Factor-D impeded 2D cell growth

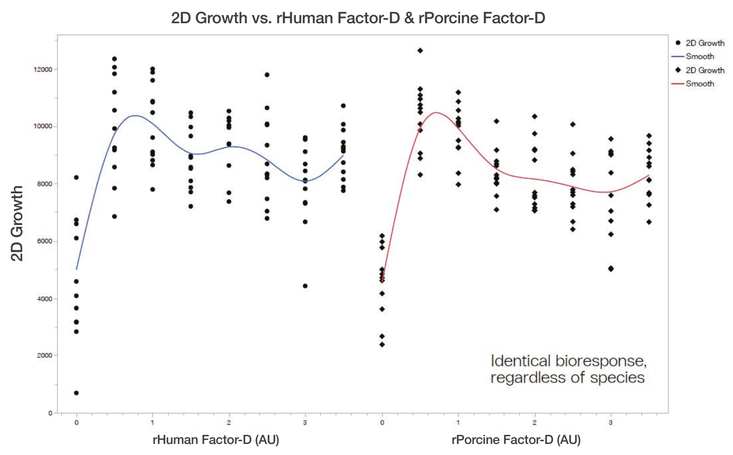


Figure 5. Follow-up experiments uncovered that Growth Factor-D is required for cell growth but only up until a specific concentration. This was demonstrated in parallel using both rHuman and rPorcine homologs.

Optimizing growth media for 3D cell culture

- 3D assays more closely replicate the conditions in a bioreactor, where cells will ultimately be grown in suspension. These assays are used to screen media at a much smaller scale, saving costs and resources.
- To further reduce costs, the team investigated the relative contributions of the four Growth Factors (A, B, C, D) on total 3D biomass.
- These experiments follow a similar workflow (Figure 6), combining DoE and dragonfly discovery to generate unique formulations and dispense media into 48-well plates.
- After 2-4 days incubation, total biomass is measured using tile scan imaging and AI image analysis.

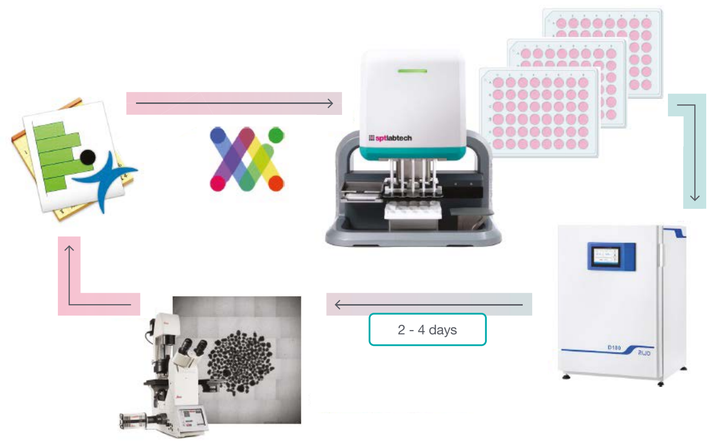


Figure 6. Iterative process used to optimize growth media for small-scale 3D cell cultures.

Initial data showed that Growth Factor-A and Growth Factor-B, and the interaction between these two supplements, had the most significant contributions. Plotting this data together in a heat map (Figure 7A) identified that both are required for a viable 3D culture, but neither are sufficient on their own.

The workflow was repeated using 10 different concentrations of both Growth Factor-A and Growth Factor-B (Figure 7B) to identify the minimum concentrations that achieve peak biomass production. These findings have since been incorporated into large scale bioreactors (3L and 50L) to achieve successful and cost-effective production at scale.

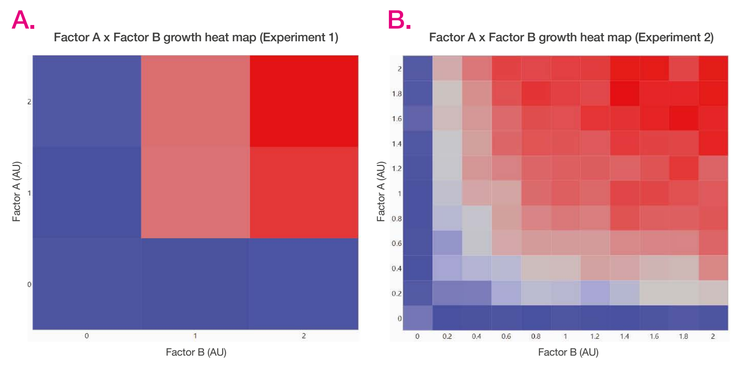
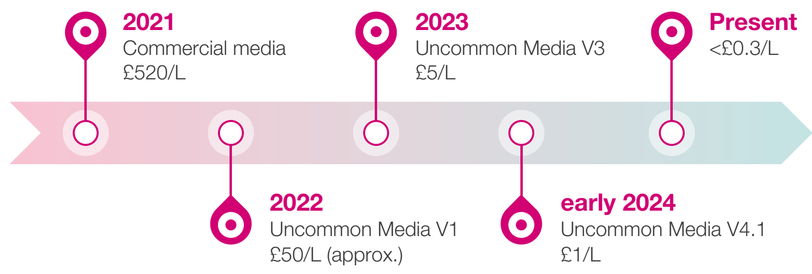


Figure 7. Heat map showing the effect of Growth Factor-A and Growth Factor-B on 3D cell culture growth using 3 (A) and 10 (B) different concentrations of each.

Conclusion

- The combination of DoE and automated liquid handling enables hundreds of high-throughput experiments, to investigate the effect of multiple factors at once, as well as the interaction between different factors.
- Uncommon have used this approach to optimize media supplement formulation for both 2D and 3D cell cultures of iPSCs.
- The resulting media is over 1000X cheaper compared to commercial media.
- This approach has also been successfully applied to optimize the composition of the RNA delivery system used to differentiate iPSCs into fat and muscle cells, further optimizing the cost efficiency of the workflow.



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