

Bioprocess intensification strategies for cultivated meat production

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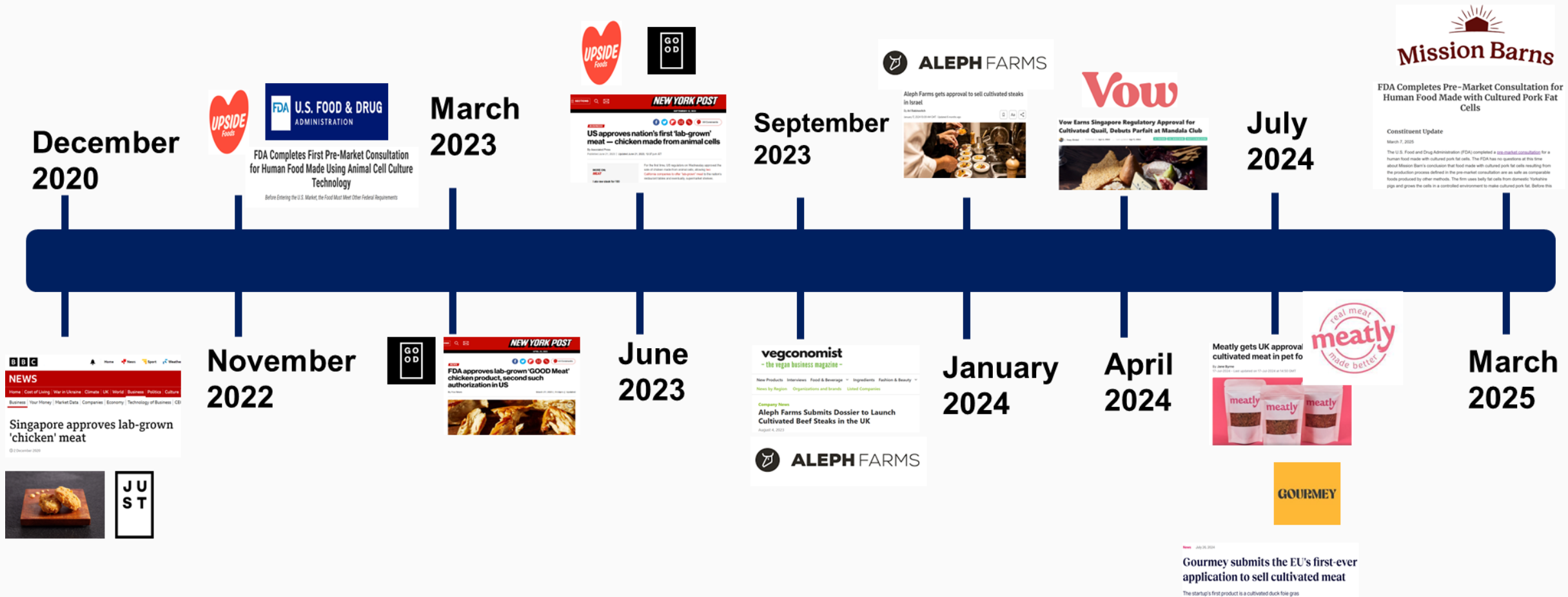
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Pathway to market



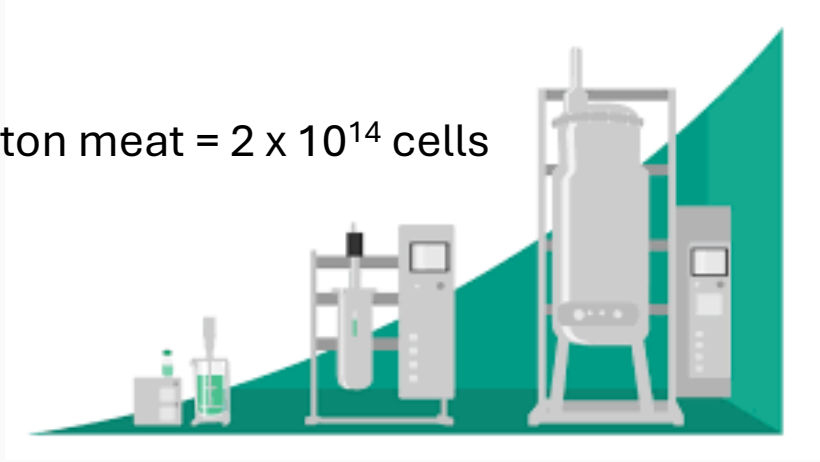
Regulatory approvals to date

COMPANY	COUNTRY OF APPROVAL	PRODUCT (SPECIES)	PRODUCT	APPROVAL DATE	AVAILABILITY	MILESTONE(S)
	Singapore & USA	Chicken	Pieces, shredded	Dec 2020 & June 2023	Not on sale currently	1 st approval (SG & USA with Upside); 1 st retail sale (SG);
	USA	Chicken	Fillet	June 2023	Not on sale currently	1 st approval (USA) (with Good Meat)
	Israel	Beef	Steak	Dec 2023	Not on sale currently	1 st Israeli approval
	Singapore, Australia & NZ	Quail	Parfait, foie gras, croquettes	April 2024 & June 2025	Multiple restaurants in Singapore & Australia	1 st AUS/NZ approval
** 	UK	Chicken	Oven baked treats (for pets)	July 2024	Not on sale currently	1 st pet food approval; 1 st sale in Europe
	USA	Salmon	Sashimi	May 2025	Multiple restaurants in USA	1 st seafood approval worldwide
** 	Singapore	Poultry	Freeze dried treats (for pets)	June 2025	For sale in Singapore since end of 2025	1 st pet food approval in Asia
	USA	Pork (fat)	Sausages, bacon, salami	July 2025	Restaurant & farmers market (San Francisco)	1 st fat approval; 1 st US retail sale
	Singapore	Chicken	Paste	Oct 2025	Not on sale currently	1 st European company to get approval
	USA	Chicken	Pieces	Oct 2025	Not on sale currently	1 st non-US company to USA approval

*<https://www.greenqueen.com.hk/believer-meats-usda-approval-lab-grown-cultivated-chicken/>

Current challenges of cultivated meat production

1 ton meat = 2×10^{14} cells



SCALE



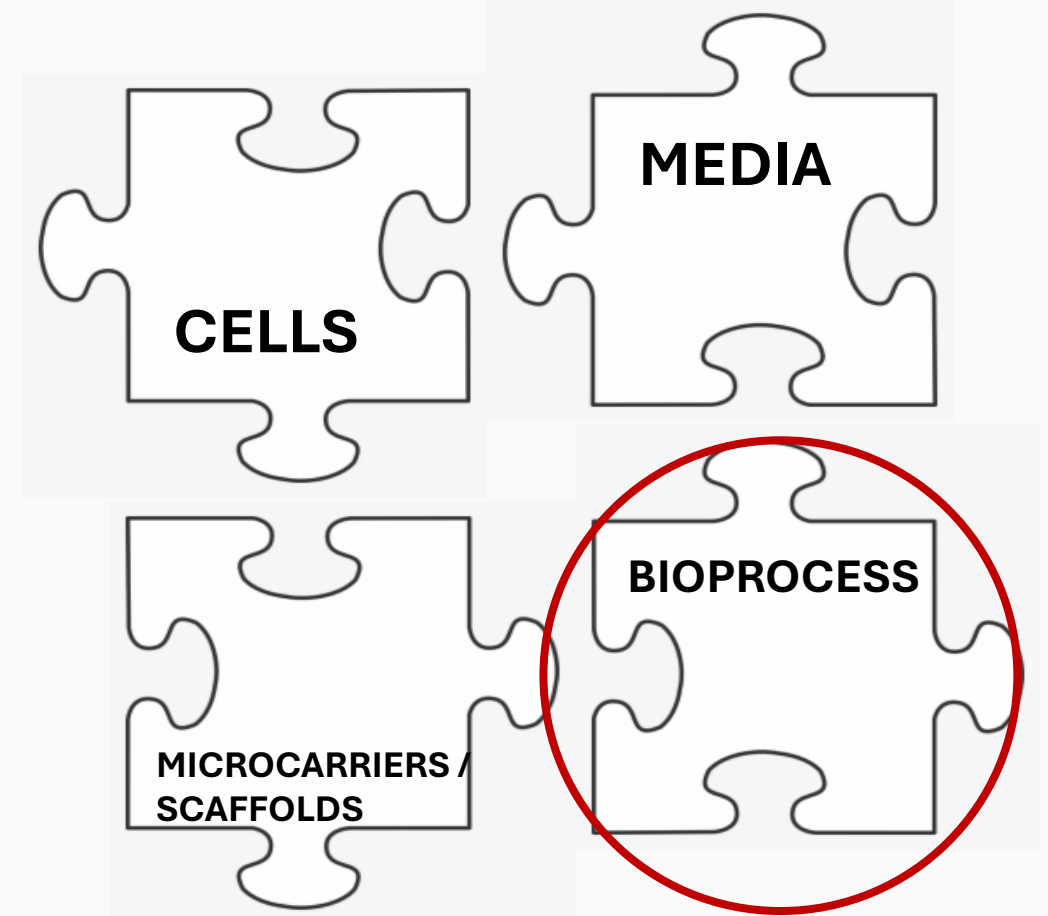
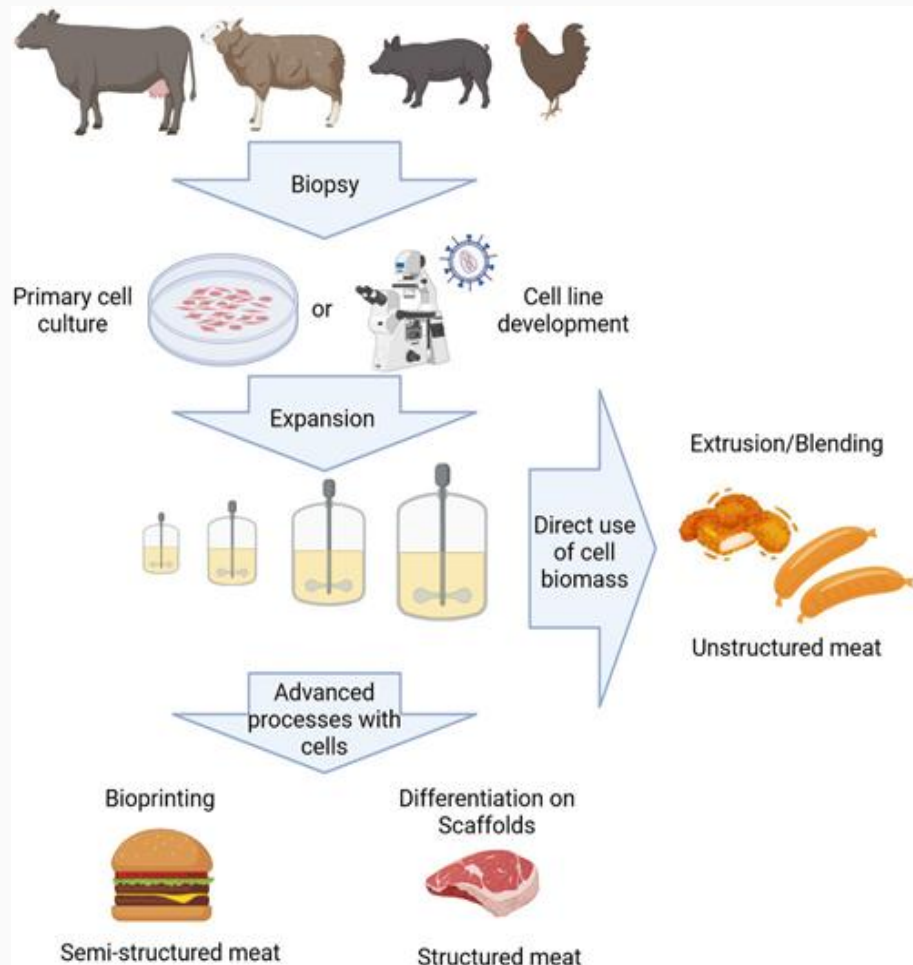
COST



World's 1st Cultivated Beef Burger – Mosa Meat (2013)



How can Cultivated Meat be produced?



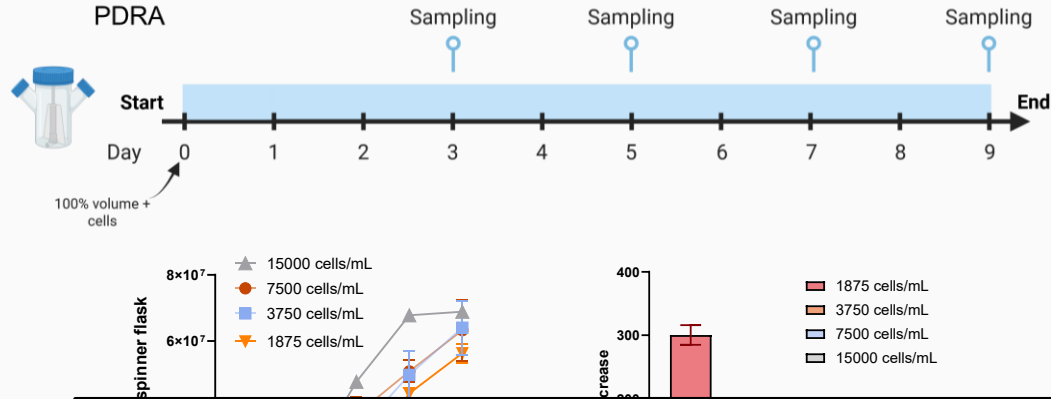
Kirsch M et al (2023) *Engineering in Life Sciences*, 23(12): e2300227

Bioprocess development: cell seeding density

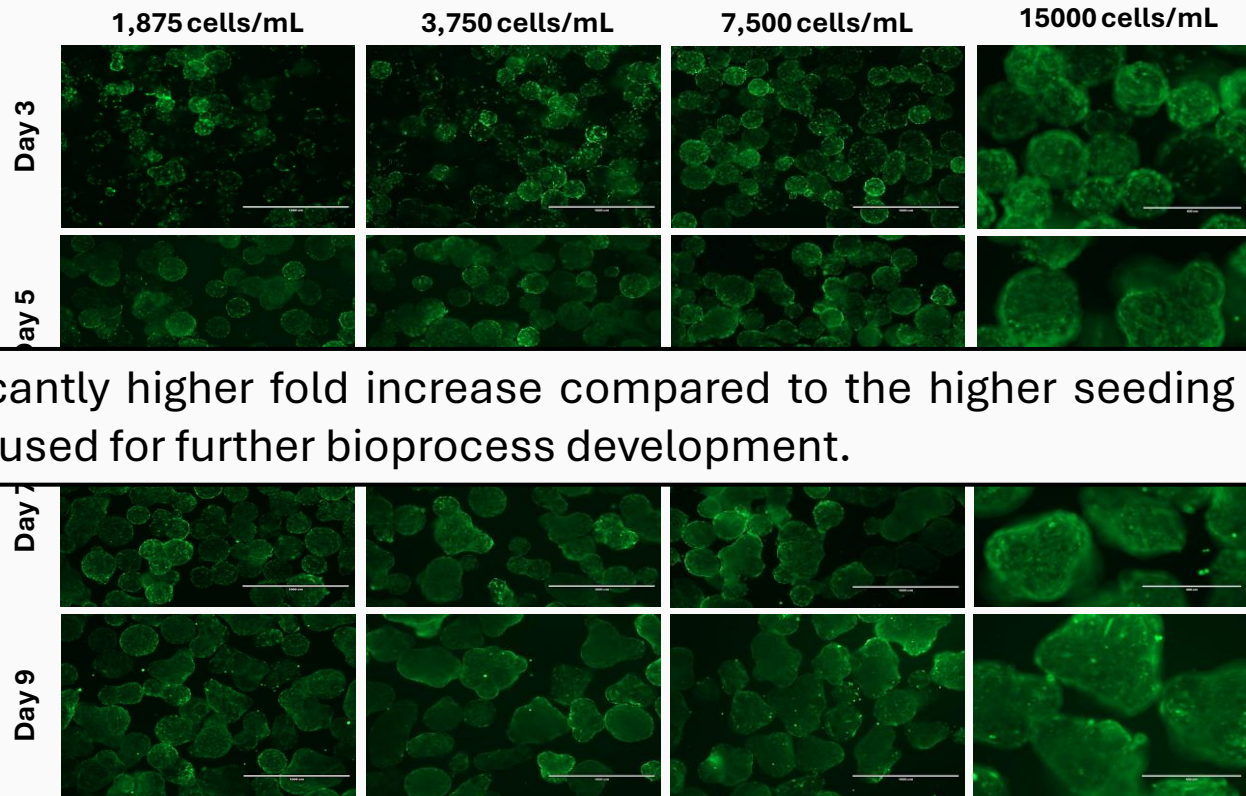
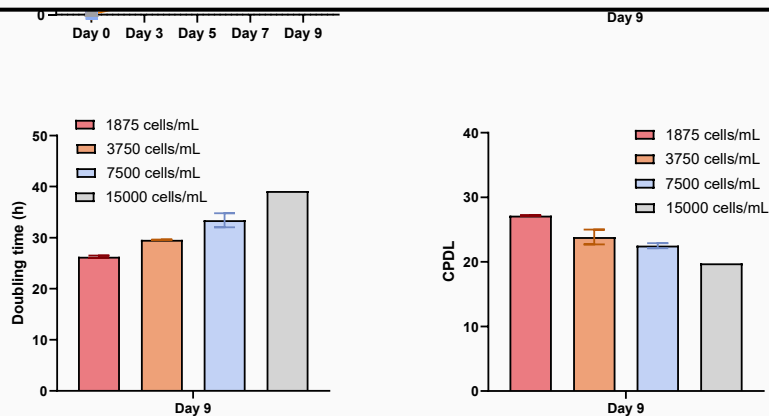


Dr Maria Laura Vieri
PDRA

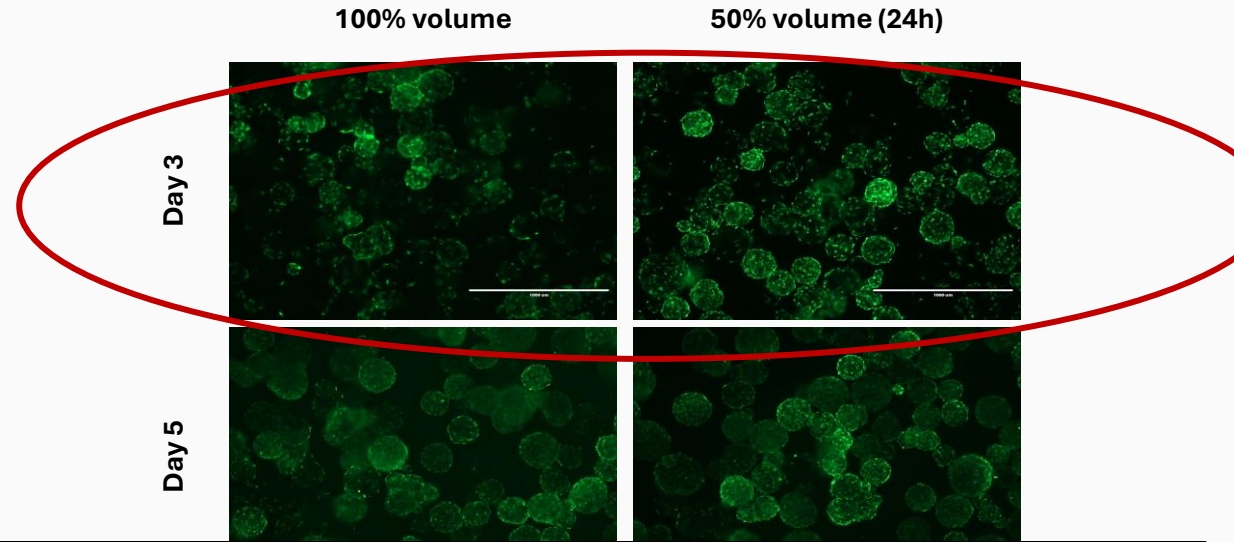
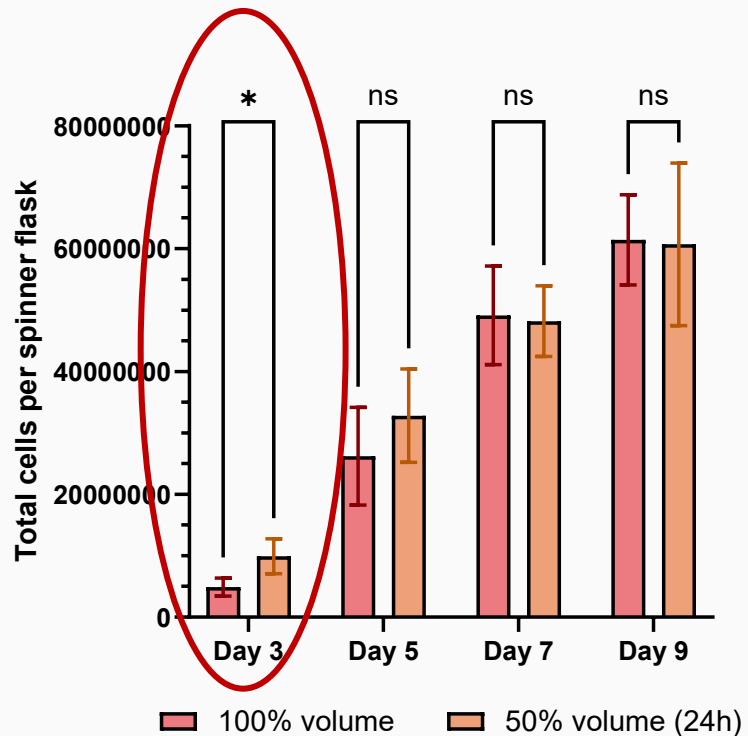
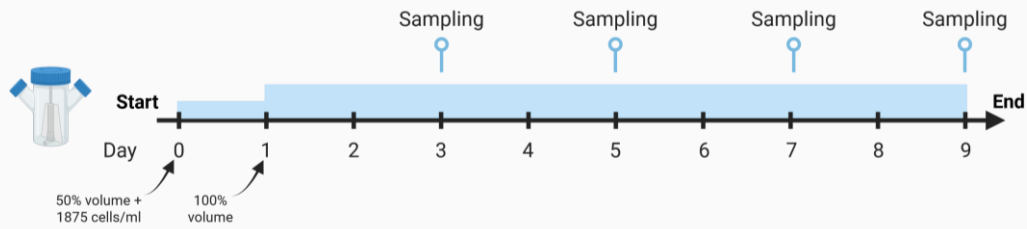
CELLS	SCAFFOLDS (e.g. Microcarriers)	MEDIA	Expansion / Differentiation
C2C12 (ATCC)	Cultispher S (Percell) (macroporous, gelatin-based)	Serum-based [Serum-free ongoing]	Expansion only



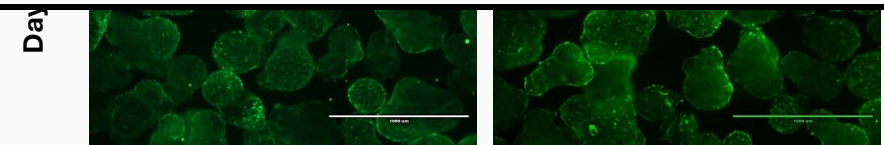
➤ The lowest seeding density tested gave a significantly higher fold increase compared to the higher seeding densities tested, so it was chosen as optimal and used for further bioprocess development.



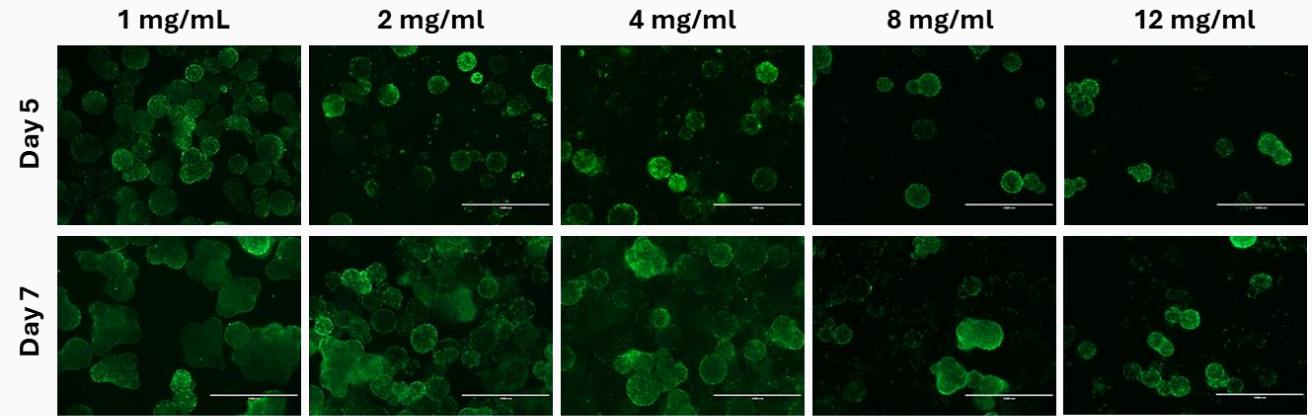
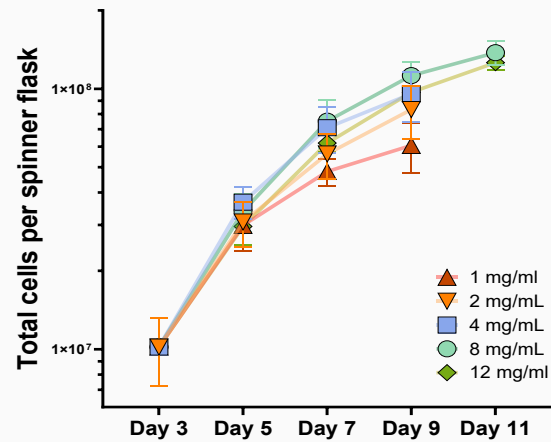
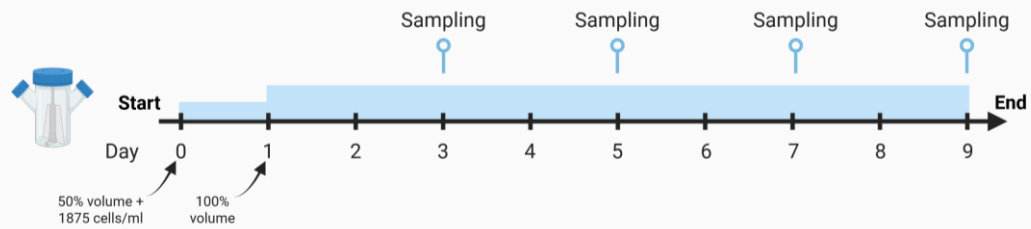
Strategies to improve cell attachment



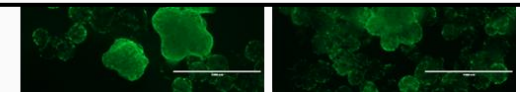
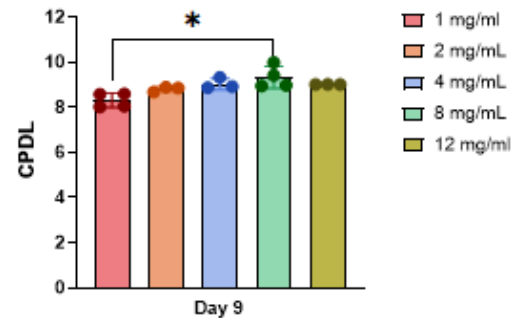
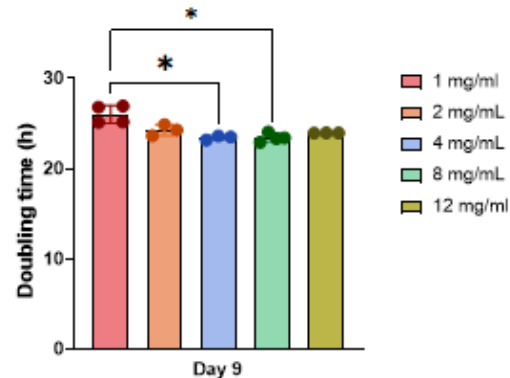
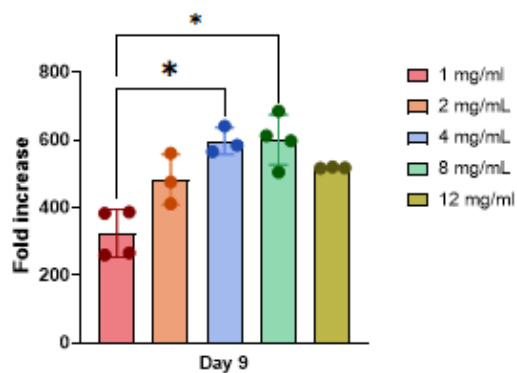
- 1) The reduction of volume at the start of culture improves cell attachment, but doesn't make a significant difference in cell growth over 9 days of culture
- 2) In both conditions tested, aggregation was present at Day 5.
- 3) However, reducing the volume of media required can lead to a reduction in manufacturing costs, especially at scale.



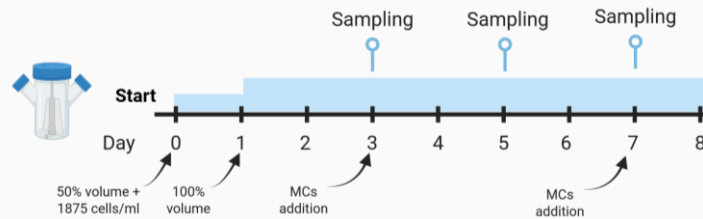
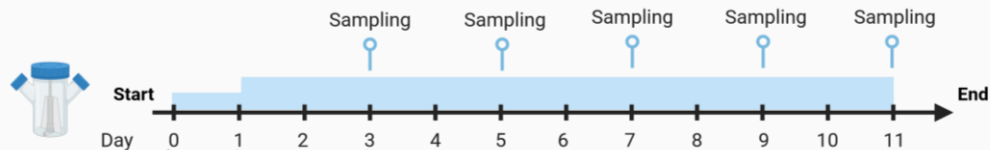
Strategy to bypass surface limitation: MC concentration



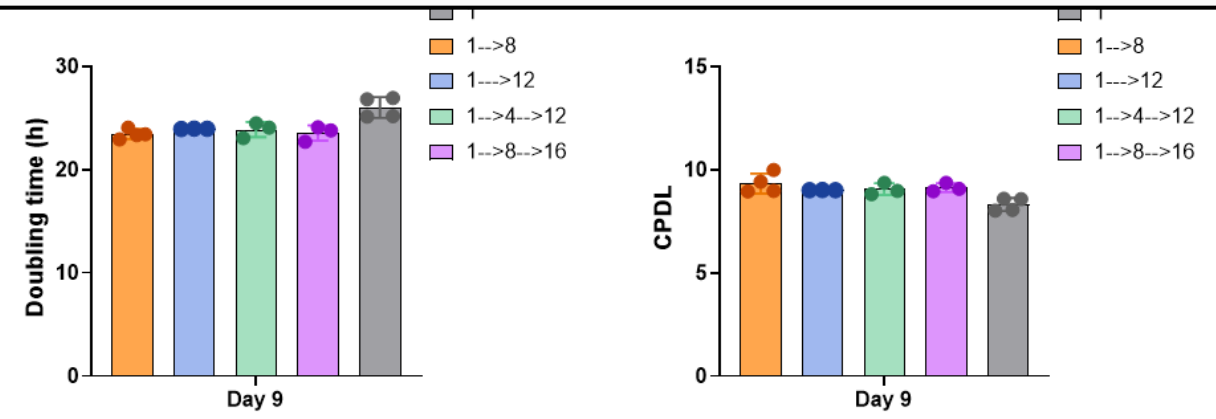
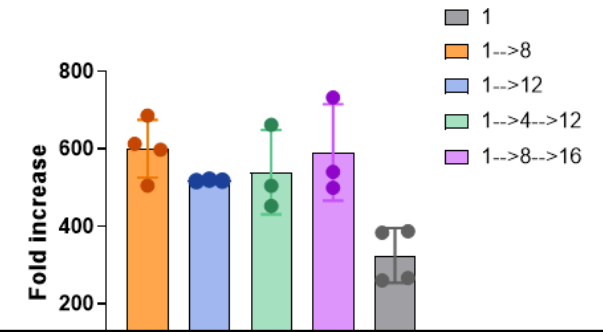
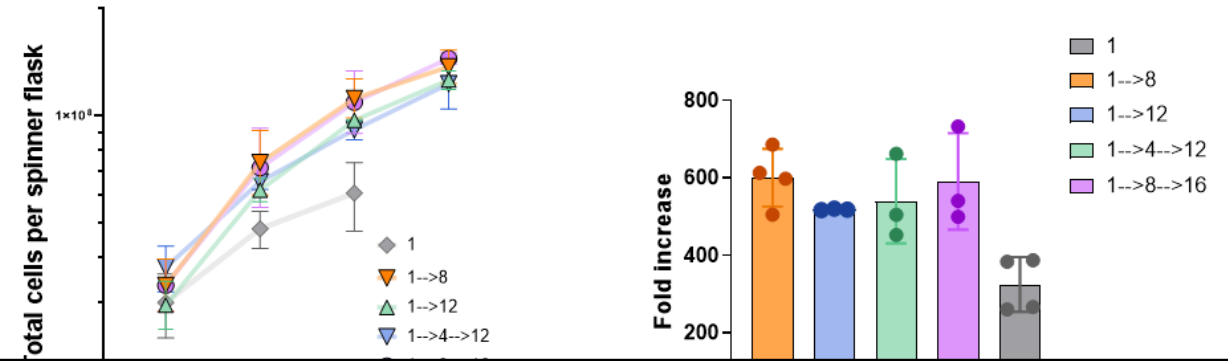
- Starting with a 4x or 8x higher microcarrier concentration resulted in significantly higher fold increases
- The level of aggregation in the higher MC concentrations was lower at Day 9 compared to the Baseline of 1 mg/mL MCs



Strategy to bypass surface limitation: MC addition (1)



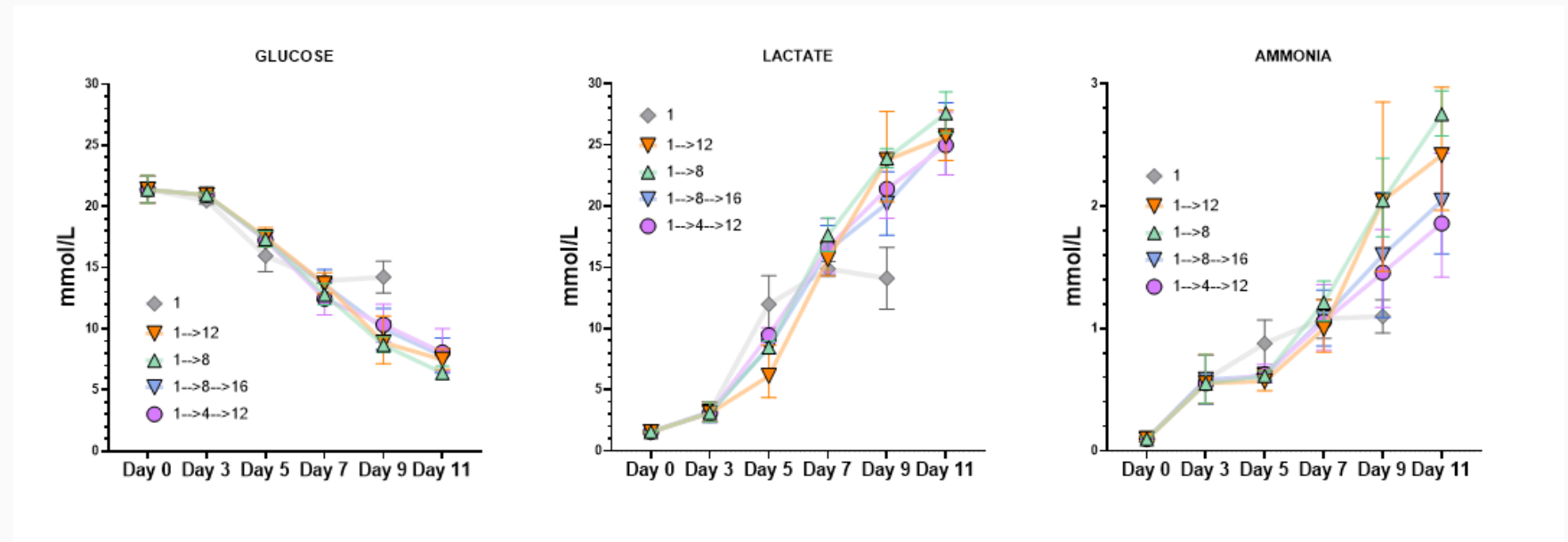
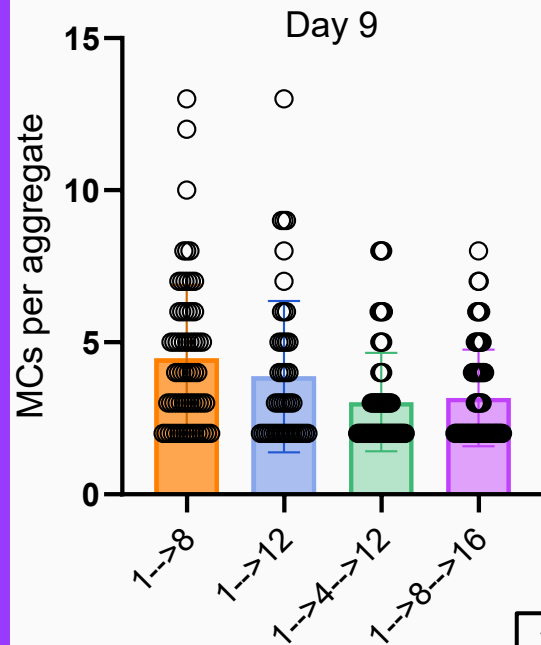
➤ No significant difference in cell growth was obtained within the culture duration tested between one or two microcarrier additions to increase surface area



Hanga MP et al (2020) *Biotechnology Bioengineering*; 117(10):3029-3039.
 Hanga MP et al (2021) *Biotechnology Bioengineering*; 118(8):3175-3186.
 Rafiq Q.A., Ruck S., Hanga M.P., et al. (2018). *Biochem. Eng. J.*; 135:11-21.

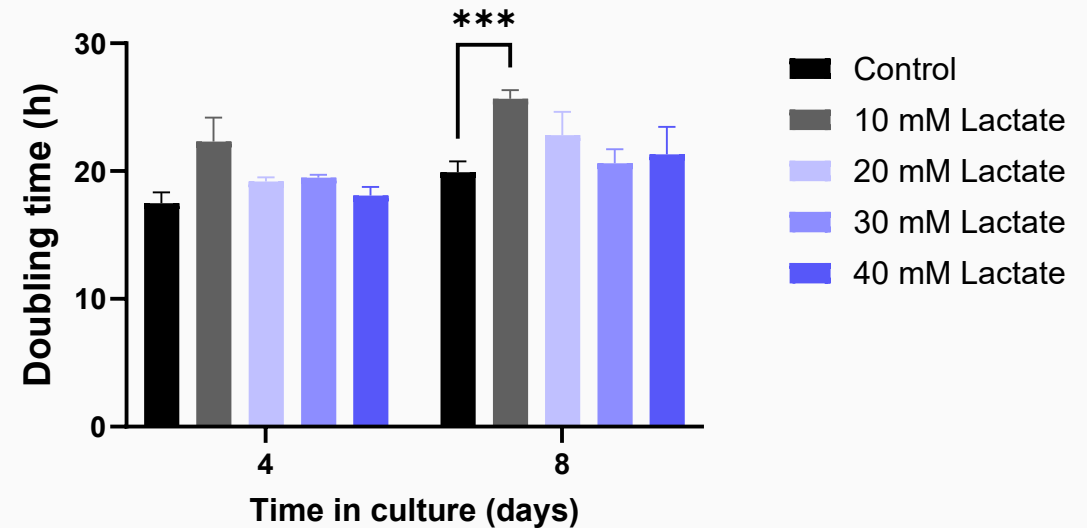
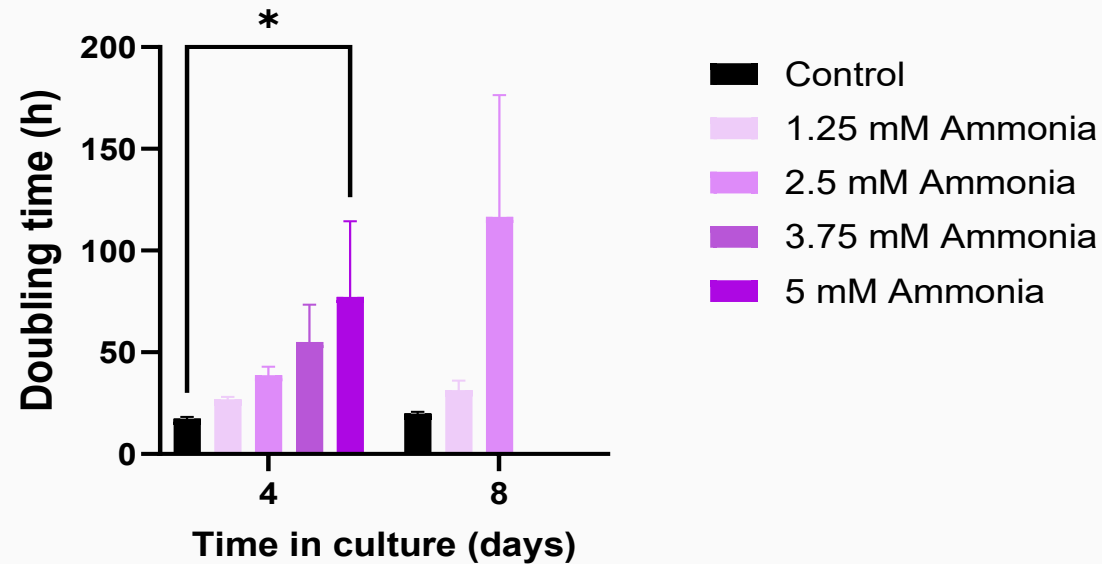


Strategy to bypass surface limitation: MC addition (2)



- Nutrient depletion and/or metabolite (e.g. lactate, ammonia) accumulation are known cell growth inhibitory factors
- Lactate concentrations of above 25 mmol/L and Ammonia concentrations of above 2 mmol/L were produced by the end of culture
- There is no published knowledge on the Lactate and Ammonia concentrations that are inhibitory to C2C12 growth

Investigate the Lactate and Ammonia effect on C2C12 growth



- Ammonia concentrations of 2.5 mM resulted in a 43.6% increase in doubling time compared to Control during the 1st passage and a 332% in the 2nd consecutive passage;
- Lactate concentrations within the range of 10 to 40 mM did not significantly affect doubling times during the 1st passage or 2nd consecutive passage compared to the Control (0 mM Lactate).
- However, we are still to investigate formulations that include various Lactate and Ammonia concentrations replicating bioreactor conditions.

Acknowledgements



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