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Sustainable Food Proteins Course
Minneapolis – October, 2025

Lecture #5
Precision Fermentation and Cell-Cultivated Proteins

Today's agenda:

- **Precision Fermentation Details**
- **Cell-Cultivated Protein Landscape**
- **Cell-Cultivated Cultures & Process**

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Simplified Precision Fermentation Process Flow

- **Engineered microorganisms to produce specific functional ingredients in a controlled and efficient manner**

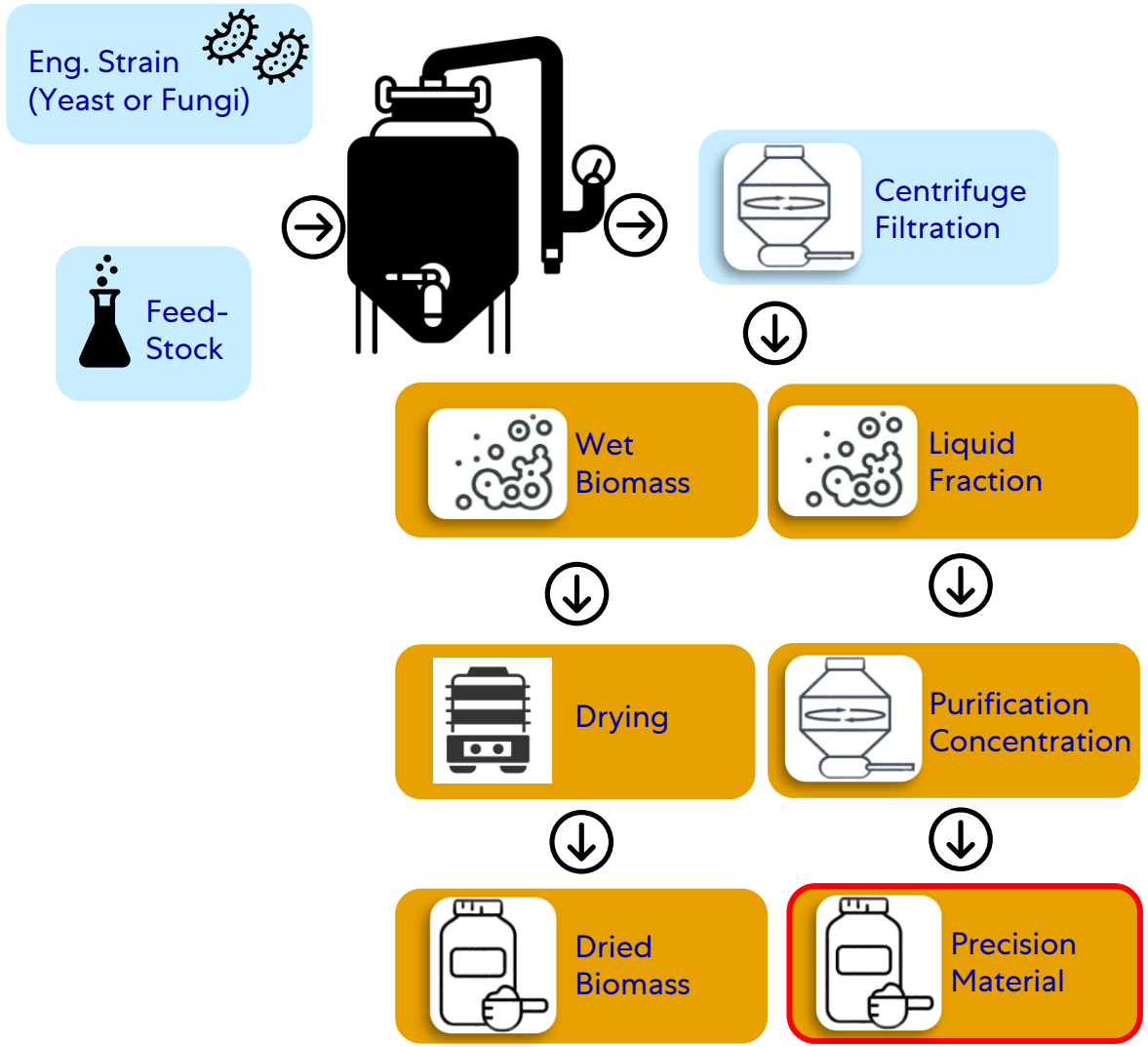
Examples of companies within the PF Landscape:

STANDING OVATION

MYCO TECHNOLOGY™

EVERY ALL PROTEIN. NO ANIMAL.

ONEGO bio



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Fermentation Types and Details

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Batch

2-5% Cell Density

20-50 g/L max

0.5-2 g/L/h

Total: 1-7 days

Definition:

Feedstock & strain added in fermentation tank – inoculate cells, growth, then all of the tank is harvested

Fed-Batch

2-10% Cell Density

20-100 g/L max

1-3 g/L/h

Total: 3-20 days

Definition:

Gas release monitored to determine when additional feedstock should be added.
Allows for increased cell density if gas can be stirred in.

Most PF

Continuous

10-20% Cell Density

100-200 g/L max

2-5 g/L/h

Total: Indefinite at steady state

Definition:

Feedstock added throughout process AND waste build-up is removed at a certain point.
This allows for increased cell density compared to Fed-Batch.

Many enabling technology companies assisting

Perfusion

20-30% Cell Density

200-300 g/L max

3-10 g/L/h

Total: Up to Months

Definition:

Feedstock added constantly & waste build-up constantly removed, creating a steady state of inputs and outputs.
Used primarily for slow growing cells.

All Cell-Cultivated

Efficiency + Complexity and Novelty

Yongky A, Xu J, Tian J, Oliveira C, Zhao J, McFarland K, Borys MC, Li ZJ. Process intensification in fed-batch production bioreactors using non-perfusion seed cultures. MAbs. 2019 Nov-Dec;11(8):1502-1514. doi: 10.1080/19420862.2019.1652075. Epub 2019 Aug 19. PMID: 31379298; PMCID: PMC6816350.
Jianfeng Xu, Xumeng Ge, Maureen C. Dolan, Towards high-yield production of pharmaceutical proteins with plant cell suspension cultures,Biotechnology Advances,Volume 29, Issue 3,2011,Pages 278-299,ISSN 0734-9750,https://doi.org/10.1016/j.biotechadv.2011.01.002.

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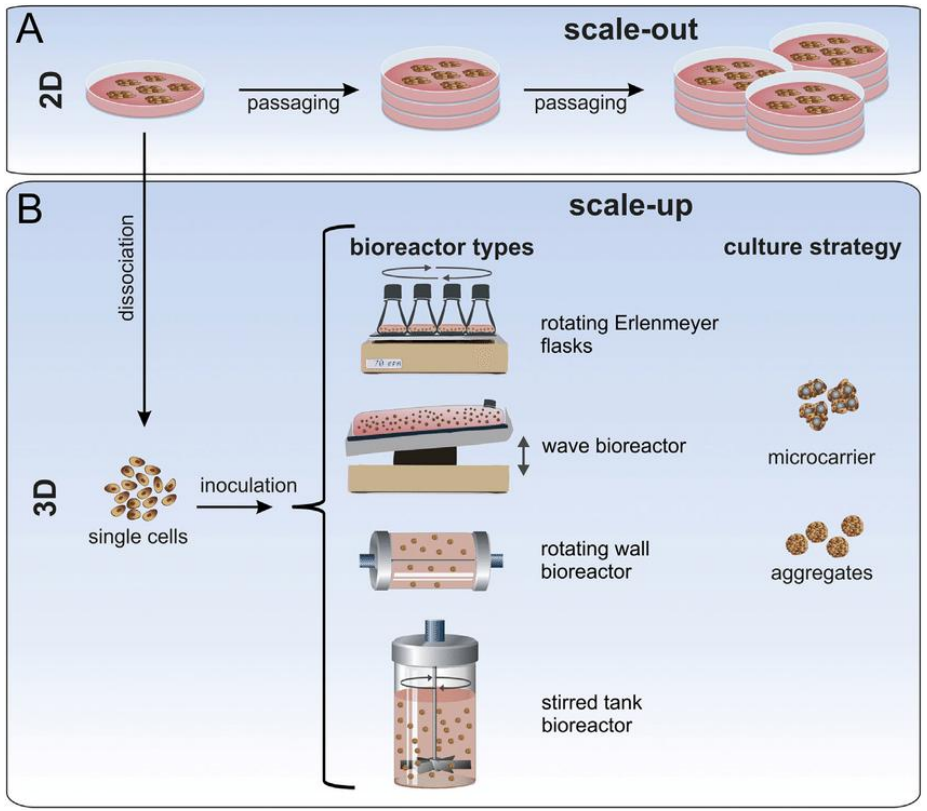
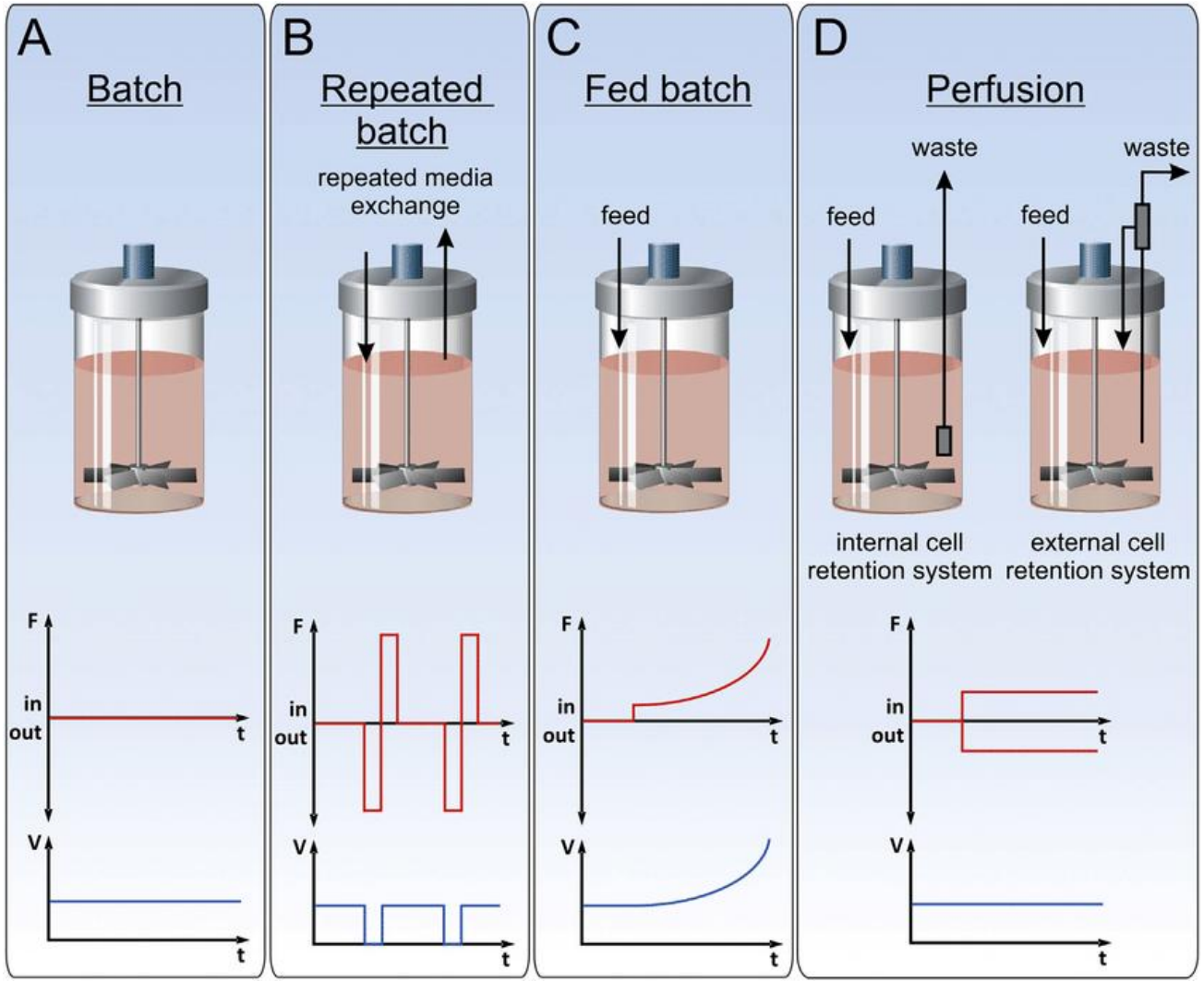
Fermentation Types Visualization





DOI: 10.1016/j.procbio.2016.09.032, Massai, Diana, Zweierdt, Robert

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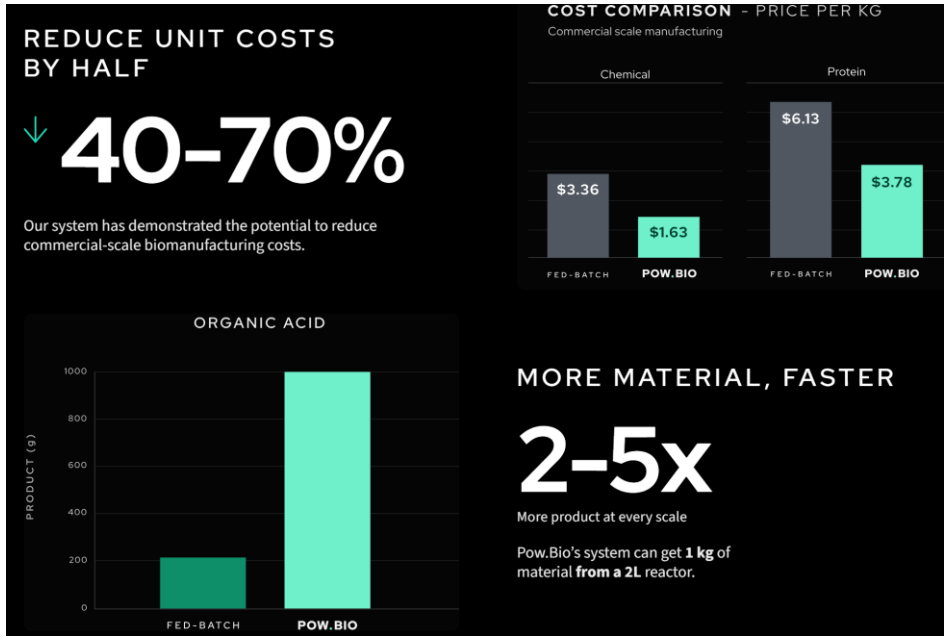
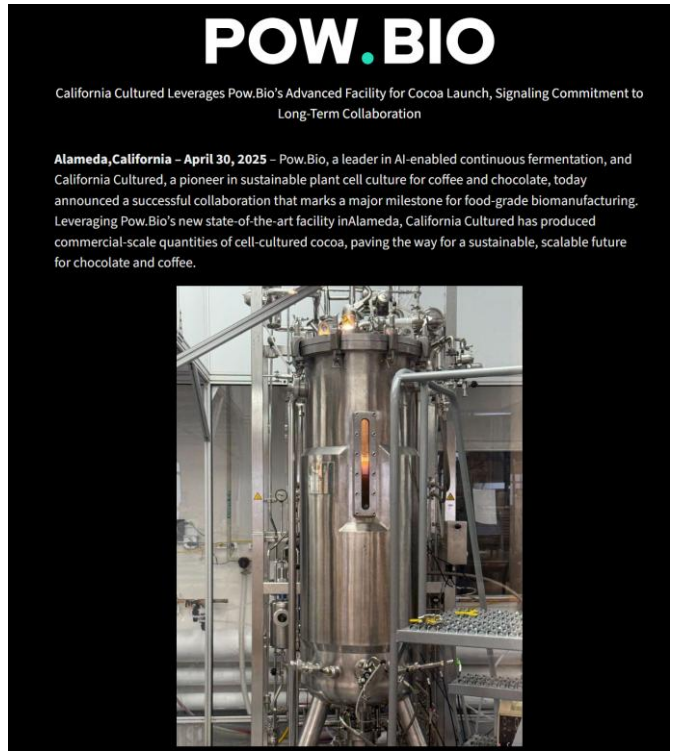
Enabling Technologies for Fermentation – Example #1

POW.BIO

Description:
Provides an AI-enabled continuous fermentation platform that dramatically boosts biomanufacturing yields and cuts unit costs by up to 50%.

Key Advantages:
Increases fermentation yields by 2-5x, and cost savings up to 50%.
Partnership with California Cultured (cell-cultivated cocoa)





https://www.pow.bio/

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Enabling Technologies for Fermentation – Example #2

Hydrosome® LABS

Description:
Uses ultrafine bubbles to boost fermentation efficiency.

Key Advantages:
Integrates into fermentation with low CapEx.
It enables 25% faster fermentation cycles and produces cleaner ingredients for texture and sweetness.

OUR ULTRAFINE BUBBLE TECHNOLOGY

Boost Fermentation Speed and Yields




Increase Biomass Yields by up to 100%

Achieve up to 2x higher peak cell biomass in fed batch fermentations. Testing has shown benefits across a number of cell lines including bacteria, yeasts and filamentous fungi for both aerobic and anerobic fermentations.

Shorter Fermentation Time

Studies using Hydrosome Labs technology fitted to existing bioreactors resulted in up to a 25% faster time to completion. Save costs through better throughput, with lower energy and labor costs and better capital utilization.

Achieve Significant Increases In Titers

Testing with Hydrosome Labs technology has shown a direct correlation between the increase in cell biomass created and Titer production. Achieve a significant increase in your target compounds, while also creating a more efficient downstream process.

Chemical Free, Easily Adapted to Your System

Our process is completely chemical free with minimal energy requirements. Our technology is designed to seamlessly integrate with your existing fermentation equipment. Bioreactors with suboptimal oxygen uptake rates (low OUR) can be easily upgraded, boosting outputs and enhancing performance.

https://hydrosomelabs.com/fermentation/

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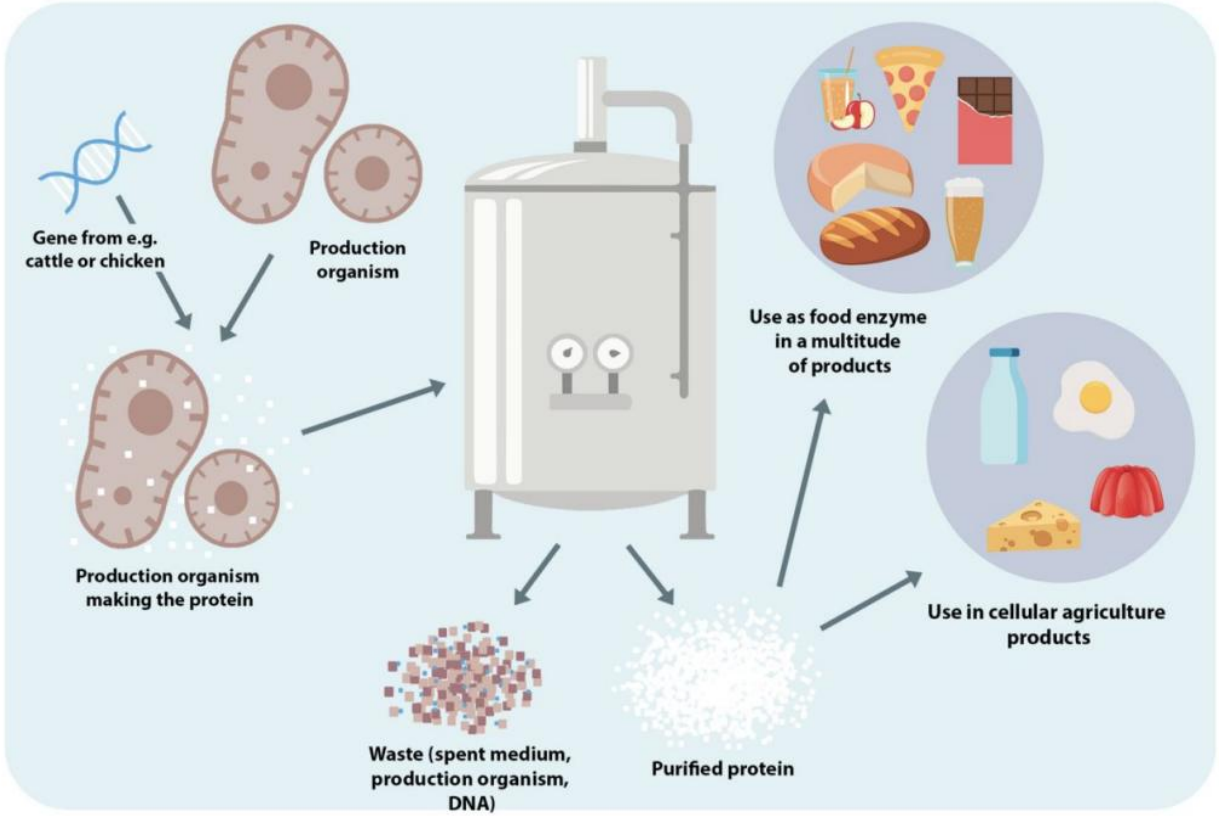
Cell Cultivated Fermentation

This type of PF is more complex, as nature identical cell are grown.

Adds upstream complexity due to cell types having slower growth, higher media requirements, & increased risk of contamination



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Mission Barns

BlueNalu

GELTOR

BOND PET FOODS™

California CULTURED

MeliBio

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Why cultivated meat?

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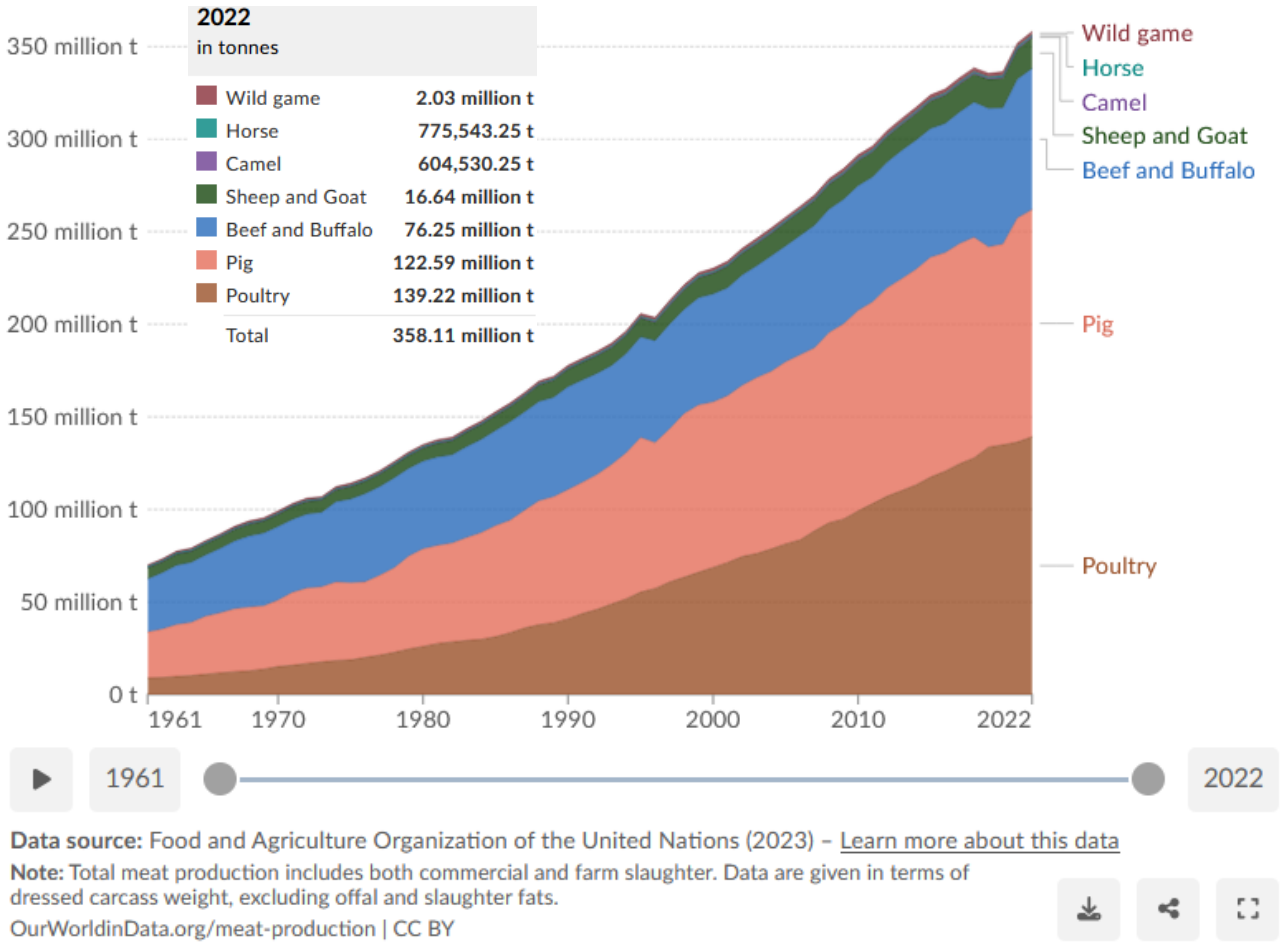
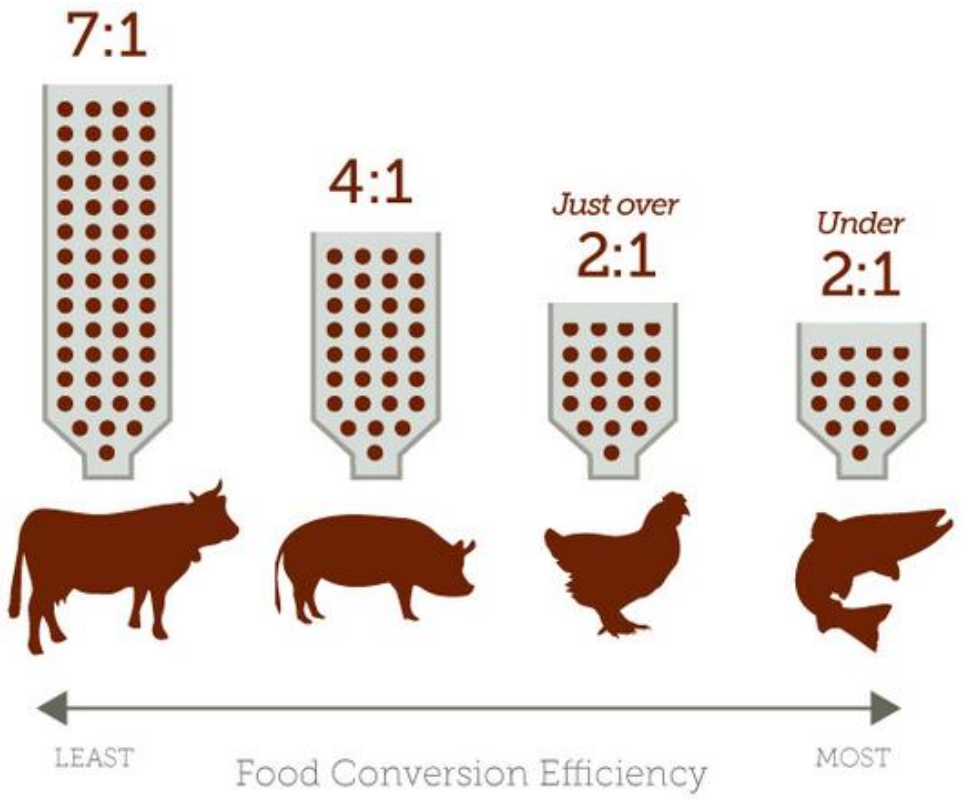
Global Meat Production by Livestock



Food Conversion Efficiency

Pounds of feed to produce 1 pound of animal protein



https://ourworldindata.org/meat-production

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Why cultivated meat?

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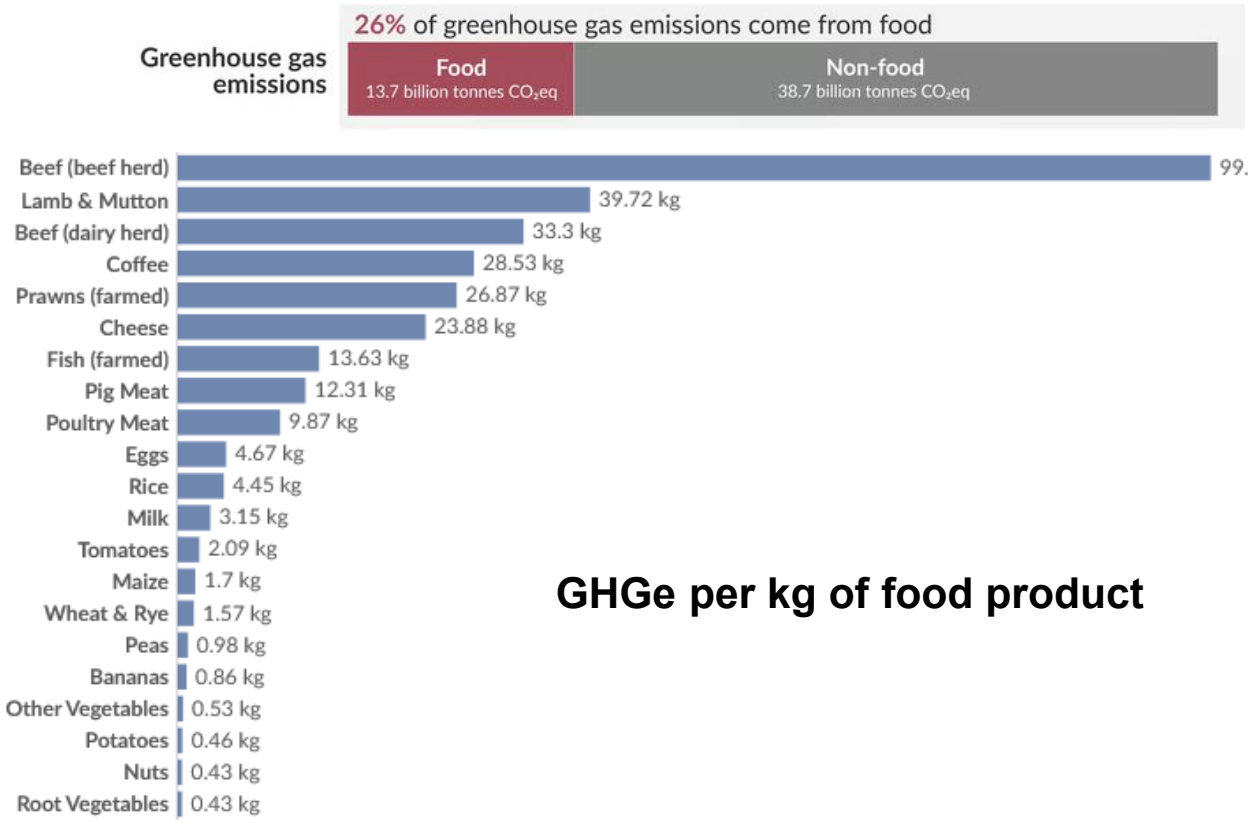
Environmental Impacts

- Livestock production uses **70%** of agricultural land but provides only **20%** of calorie intake.



GHGe per kg of food product

https://ourworldindata.org/environmental-impacts-of-food

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Protein from Animal Sources

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Nutritionally, why doe we consume animal products?

COMPLETE PROTEIN

(all 9 essential amino acids present)

Typical Meat = Muscle + Connective Tissue + Fat

Approximate composition:

- 70% water
- 20% protein
- 9% fat
- 1% ash

Content (%)	Beef	Poultry
Moisture	71.38[b]	75.03[a]
Protein	16.01[b]	17.35[a]
Fat	7.93[a]	5.12[b]
Ash	0.79[b]	0.86[a]

a-b means in same row bearing different superscript letters are significantly different ($P \leq 0.05$). n=9

J.G. Sebranek, CHEMICAL ANALYSIS | Raw Material Composition Analysis, in Encyclopedia of Meat Sciences, Academic Press, 2014, https://doi.org/10.1016/B978-0-12-384731-7.00053-2.

Hammad H, Ma M, Hydamaka A, Elkhedir A, Jin G, et al. 2019. Effect of Freeze and Re-freeze on Chemical Composition of Beef and Poultry Meat at Storage Period 4.5 Months. J Food Process Technol. 10: 791. 10.4172/2157-7110.1000791

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Cultivated meat manufacturing

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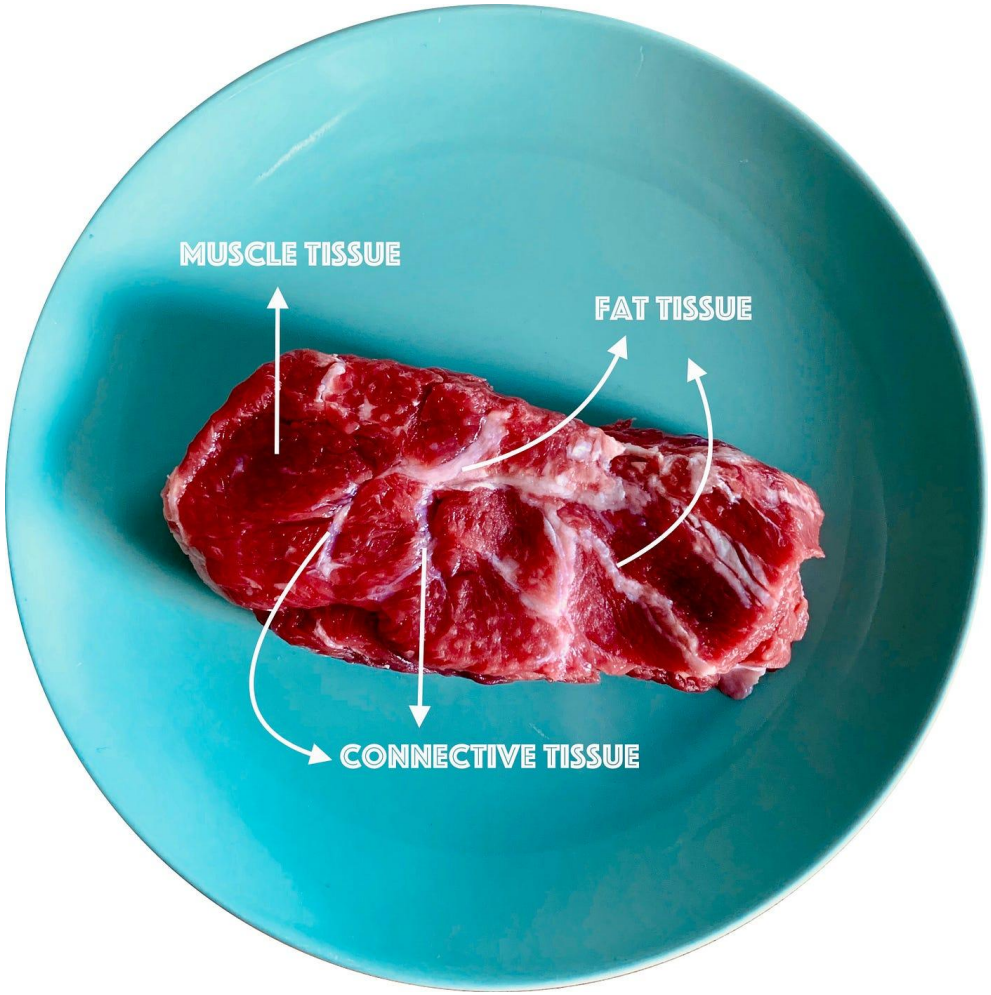

https://medium.com/cookemistry/beef-101-part-i-ee877bc7bf8c

Mimicking animal-derived meat

Meat component	Initial function	Culinary contribution to the final meat	Cultivated meat component
Muscle	Contraction/ movement	Texture, biomass	Muscle cells
Fat	Storage of energy participates in signaling	Contribution of flavor substances, juiciness, texture	Adipocytes
Connective Tissue	Structure, Support, protection	Texture, biomass	Non/Fibroblasts
ECM	Structure, tissue homeostasis/cell-matrix interactions	Texture, juiciness	Natural and synthetic Scaffolds
Blood vessels	Oxygen and nutrients supply, metabolite removal	Color, flavor	Recombinant heme proteins/food dye/ beet juice

Kirsch M, Morales-Dalmau J, Lavrentieva A. Cultivated meat manufacturing: Technology, trends, and challenges. *Eng Life Sci*. 2023; 23:e2300227. https://doi.org/10.1002/elsc.202300227

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Cultivated meat manufacturing

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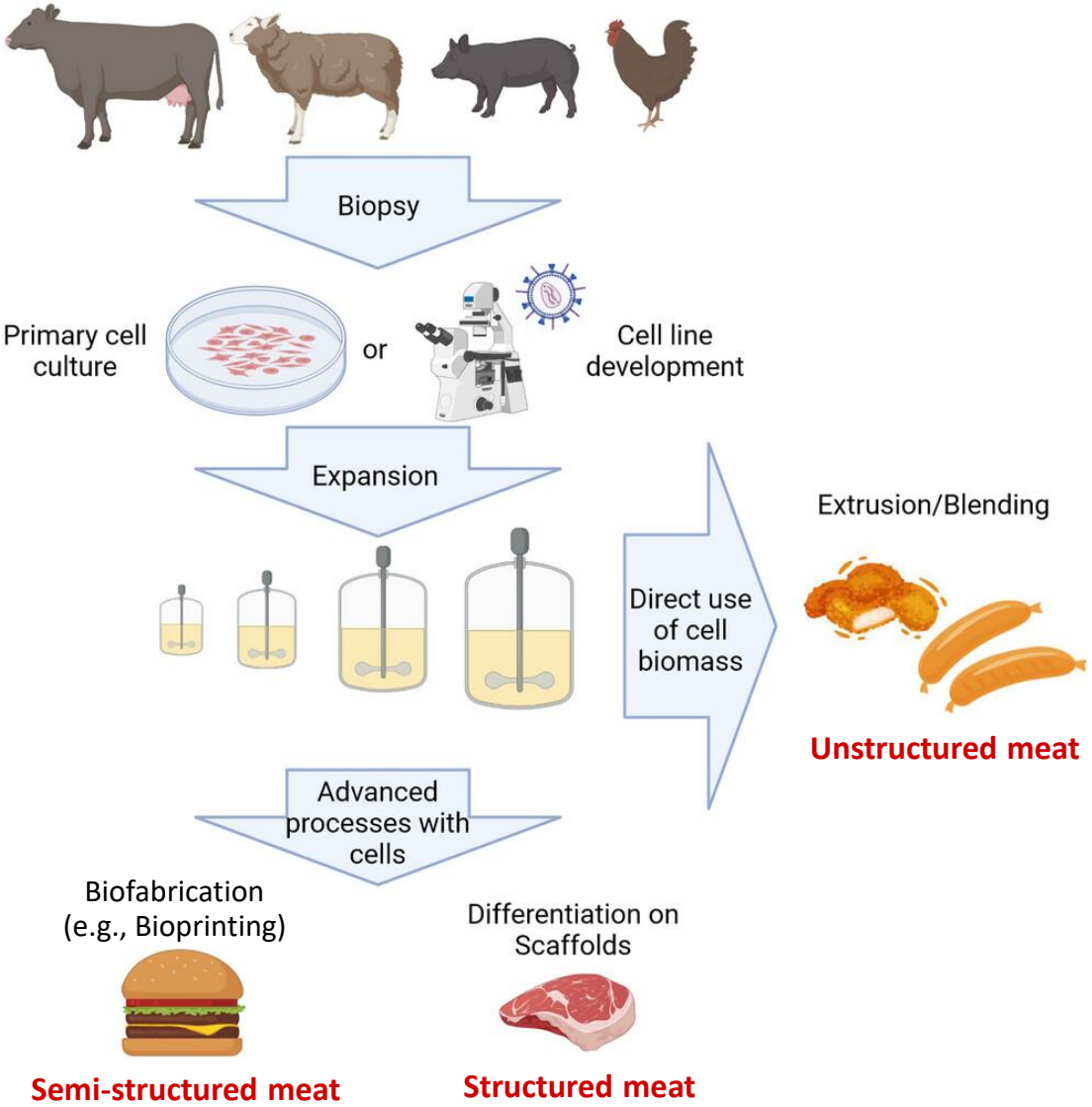

Cells
- Source
- Storage
- Optimization for large-scale
- Non-gm immortalization
- Chemically induced pluripotency

Expansion
- Bioreactors
- On-line monitoring
- Harvesting
- Degradable/ Edible microcarriers

Scaffolds
- Microcarriers
- Porosity
- Vascularization
- Biodegradable materials
- Tunable properties

Media Composition
- Animal supplements
- Pharmagrade/Feed grade
- Recycling/ Methabolites withdr.
- Differentiation triggers
- Recombunant growth factor production

Differentiation
- Bioreactor Type
- Shear stress (mechanoreceptors)
- Scale-up
- Oxygen supply
- Automatization
- Monitoring of differentiation

Kirsch M, Morales-Dalmau J, Lavrentieva A. Cultivated meat manufacturing: Technology, trends, and challenges. *Eng Life Sci*. 2023; 23:e2300227. https://doi.org/10.1002/elsc.202300227

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Cultivated meat manufacturing: Cells

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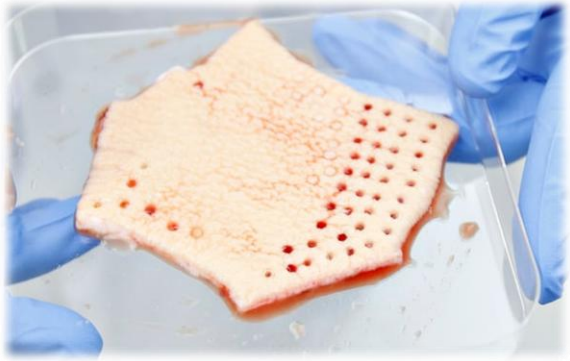
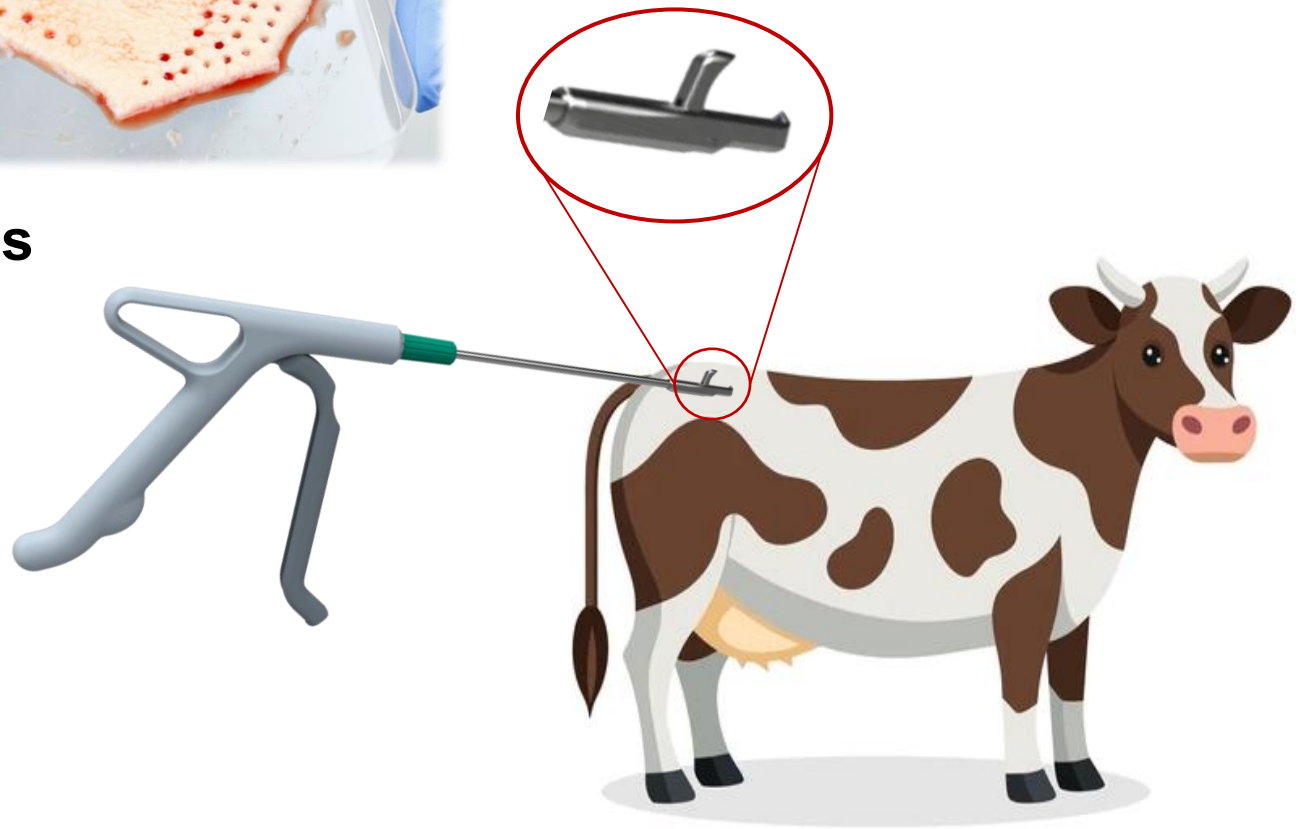
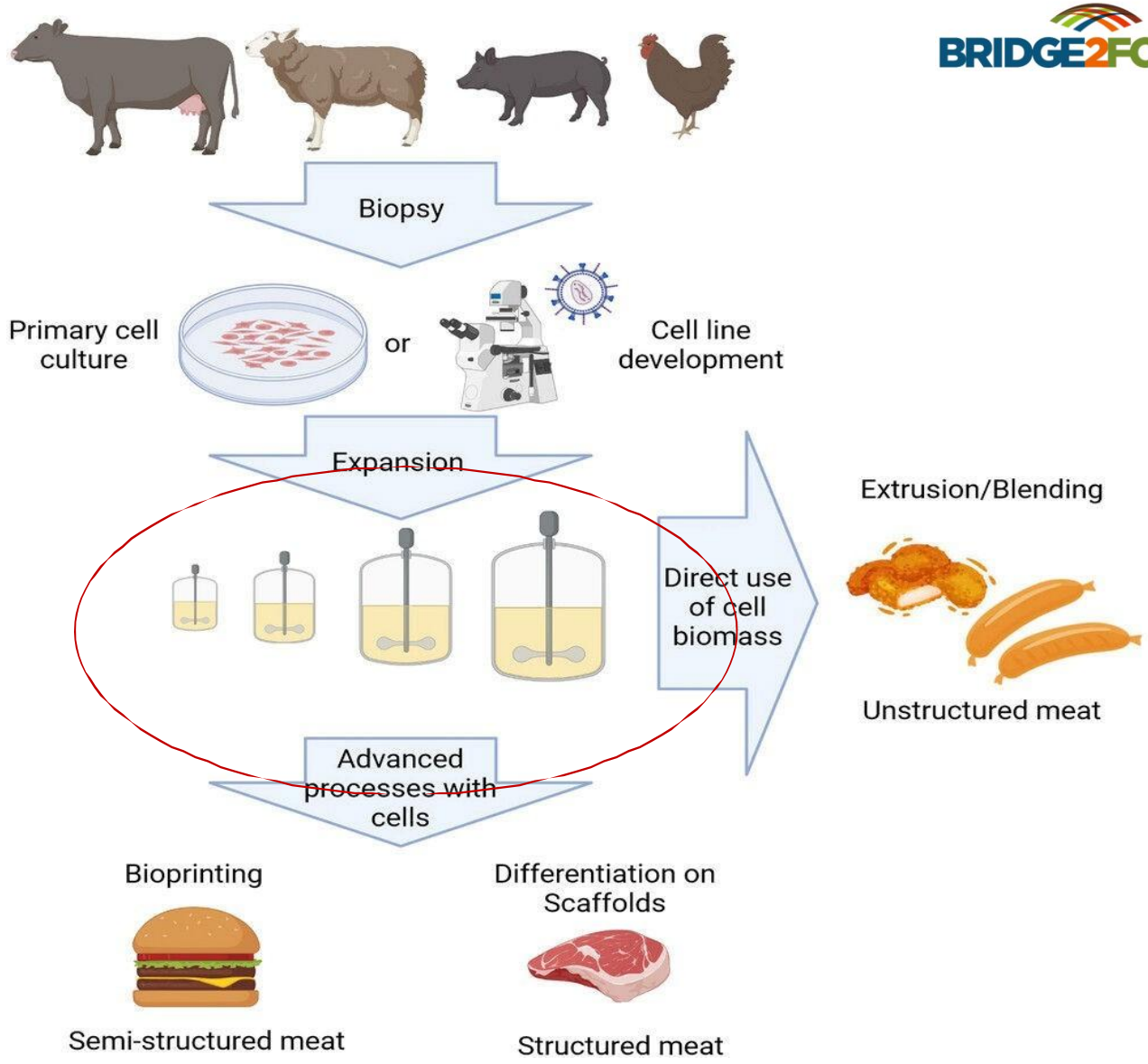


- **Primary cells vs immortalized cell lines**
- Stem cells vs specialized cells
- Adherent vs suspension cell culture



Why focus on cell culture medium?

- Biggest contributor to cost of CM production
  - 31-99% based on various assumptions
  - Variability in estimates
  - References available



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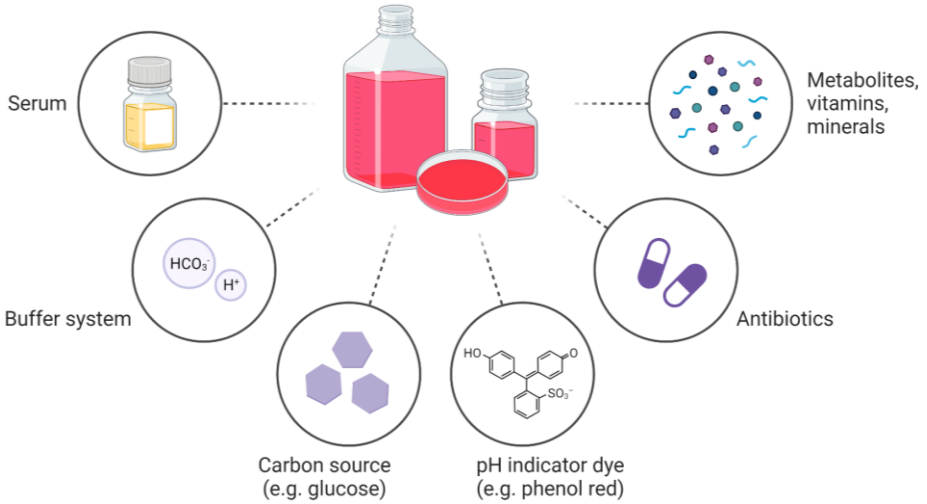
Cultivated meat manufacturing: Cell culture medium

Culture medium = Food for cells to be able to thrive *in vitro* and perform their functions



Component	Examples	Function
Basal nutrients	Amino acids, glucose, vitamins, minerals, salts	Provide essential building blocks for cell metabolism and growth
Serum/serum alternatives	A complex mixture of growth factors, hormones, amino acids, lipids etc. Fetal bovine serum (FBS) is traditionally used but chemically-defined alternatives (e.g., plant-based hydrolysates, recombinant proteins) desired for CM.	Promote robust cell proliferation and long-term cell maintenance
Growth factors (GF) and hormones	Insulin, FGF (fibroblast GF), IGF (insulin-like GF), TGF-β (transforming GF)	Regulate cell function, proliferation, and differentiation
Lipids and fatty acids	Oleic acid, linoleic acid, cholesterol	Support membrane integrity and metabolic function
Buffering agents	Sodium bicarbonate, HEPES, phenol red	Regulate pH
Antibiotics	Penicillin-streptomycin, amphotericin B	Prevent contamination

https://www.biorender.com/template/components-of-cell-culture-media

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Cultivated meat manufacturing: Cell culture medium

- Cost and economic impact
- Proliferation vs differentiation media
- Pharma vs food grade
- Stability (storage and scale)
- Regulatory considerations
- Waste treatment and recycling

Author (year)	Summary	Assumptions	Production goal ($kg yr^{-1}$)	Media type and cost estimate	Baseline media cost (US l^{-1})	Bioreactor	Animal model	Cell type	Maximum cell density ($cells ml^{-1}$)	Cell doubling time (h)	Baseline cost of product (US kg^{-1})	Media cost (%)
Risner et al.[21] (2020)	A preliminary TEA	Food grade Total cell volume for each bioreactor set equal to bioreactor volume	121,000,000	Essential 8 Vendor prices from ref. 26.	377 (scenario 1 in ref. 26)	20,000 l STR, fed-batch	Beef	Bovine MSCs, growth variables based on human embryonic stem cells	Scenario 1: 1×10^7 Scenario 2, S3: 9.5×10^7 Scenario 4: 2×10^8	Scenario 1: 24 Scenario 2, S3: 16 Scenario 4: 8	400,000	>99%
Humbird[19] (2021)	TEA and in-depth analysis if raw material supply chain is also scaled	Pharma-grade Scaled raw materials industries Metabolic engineering to reduce density inhibition CO_2 inhibition at large volumes	6,800,000 in a 100,000,000 market	Defined, serum-free Regression and bulk prices to estimate component costs at scaled production	3	20,000 l STR, fed-batch or 2,000 l STR, perfusion	Mammalian	General mammal, growth variables based on CHO cells	8.6×10^7	24	Fed-batch: 37 Perfusion: 51	60%
Vergeer et al.[24] (2021)	Current and projected (to 2030) TEA using data from seven CM companies and seven suppliers	Incorporates proprietary data from CM companies Food grade	10,000,000	Defined, serum-free Vendor prices from ref. 26 and supplier quotes	High: 537.3 Mid: 126.2 Low: 16.6	10,000 l STR and 2,000 l, perfusion with scaffolds, semi-continuous	Unspecified	Industry data averages	5×10^7	30	High: 22,421 Mid: 1,708 Low: 150	>99%
Ashizawa et al.[22] (2022)	Examines how insect cells can reduce TEA costs, adapting the model from Risner et al.[21]	Total cell volume per bioreactor set equal to bioreactor volume Food grade	121,000,000	Yeastolate–Primatone medium or Scheider's *Drosophila* medium Vendor and bulk pricing	Yeastolate–Primatone: 28.88 *Drosophila*: 13.65	20,000 L STR, fed-batch	Insect	Lepidopteran (Sf-9 and Hi-Five) or *Drosophila melanogaster* (S2) insect cell lines	Lepidopteran: 2×10^7 S2: 3.01×10^7	Lepidopteran: 22.72 S2: 38.5	Lepidopteran: 4193 S2: 6426	>99%
Garrison et al.[23] (2022)	Based on Risner et al.[21] and Specht[26], additional fixed, operational and labour costs	FGF2 and TGFβ alternatives Improved media Food grade	560,000	Essential 8 Vendor prices from ref. 26	3.74 (scenario 5 from ref. 26)	20,000 l STR, expansion and 30,000 l STR, differentiation	Unspecified	Unspecified	Unspecified	Unspecified	63.69	27.90%

Goodwin CM, Aimutis WR, Shirwaiker RA. A scoping review of cultivated meat techno-economic analyses to inform future research directions for scaled-up manufacturing. *Nat Food* **5**, 901–910 (2024). https://doi.org/10.1038/s43016-024-01061-3

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Design criteria for CC media

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- **Constraints/nice to have**
  - Must be cheap! (no serum)
  - Cruelty-free
  - Ideally chemically defined
- **Must-do**
  - Maintain cellular physiology
  - Keep cells proliferating
  - Cells must be able to differentiate (if applicable)

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Stem cells: connection with cultivated meat?

- Not cancer
- Can be maintained in cell culture for a long time
- CM media “inspired” by stem cell media!
- Lipid metabolism is important in stem cells
  - Important from the perspective of
    - Understanding cell physiology of cells for CM
    - Design of media

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Cultivated meat manufacturing: Bioreactors

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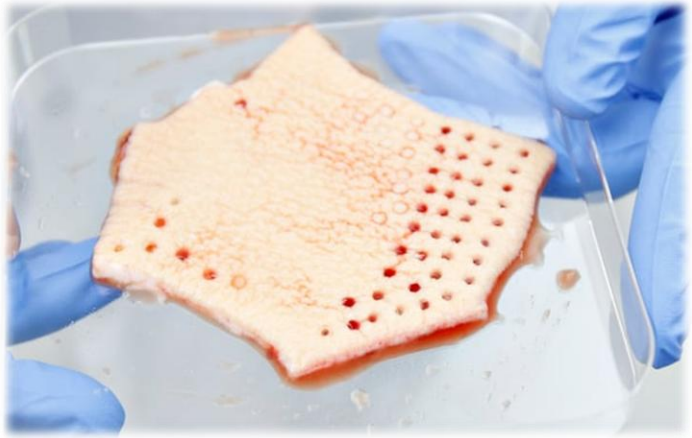

Cells at source: tens to thousands

Bioprocessing

Muscle cells in 1 kg wet meat = 2.9 x 10^{11} ***(rough estimate)***

Allan SJ, De Bank PA, Ellis MJ. 2019. Bioprocess design considerations for cultured meat production with a focus on the expansion bioreactor. *Frontiers in Sustainable Food Systems*. 3:44. https://doi.org/10.3389/fsufs.2019.00044

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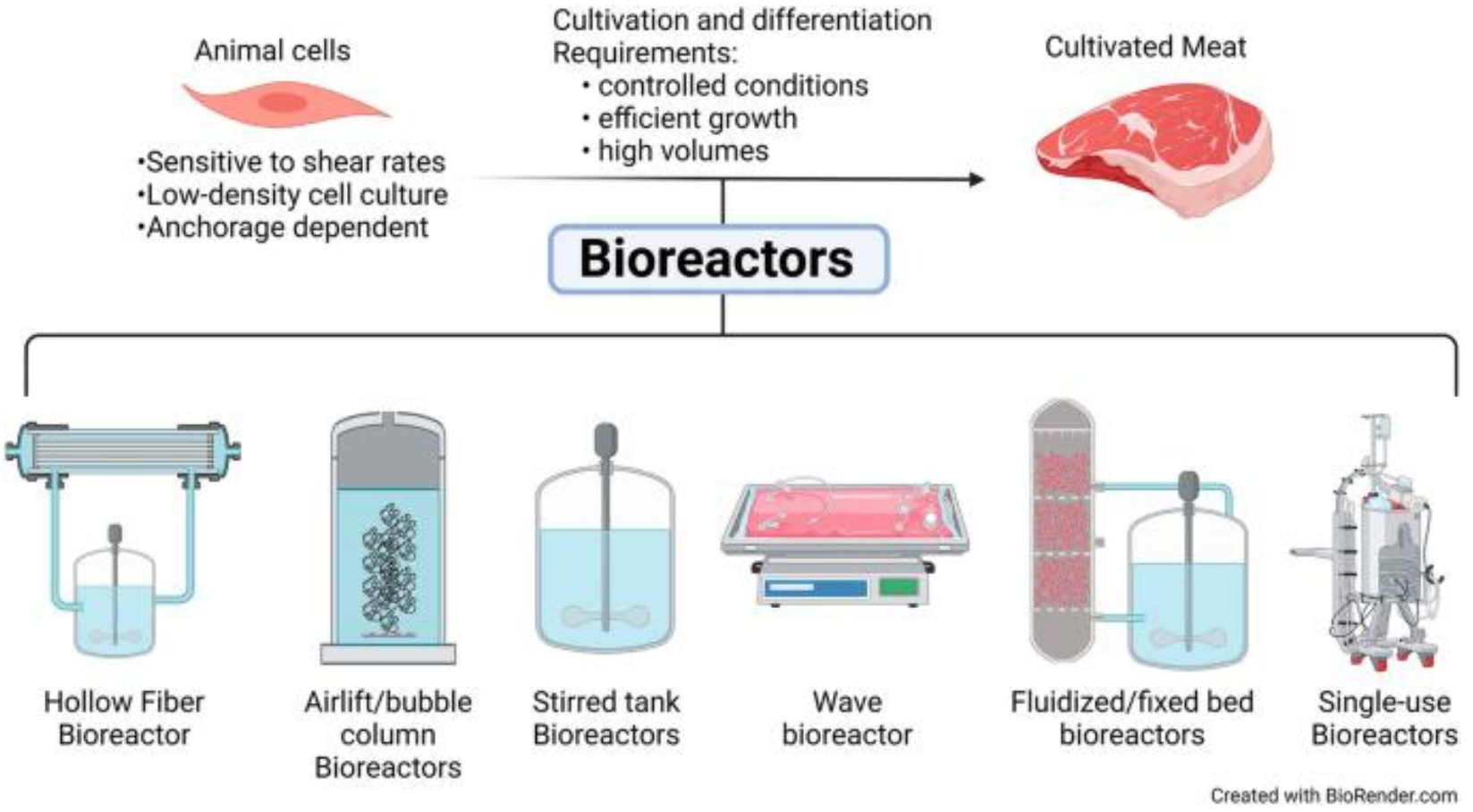
Cultivated meat manufacturing: Bioreactors

Typical lab scale adherent cell culture in flasks



Porto de Souza Vandenberghe, L. *et al.* (2024). Bioreactors for Cultivated Meat Production. In: Soccol, C.R., Molento, C.F.M., Reis, G.G., Karp, S.G. (eds) Cultivated Meat. Springer, Cham. https://doi.org/10.1007/978-3-031-55968-6_6

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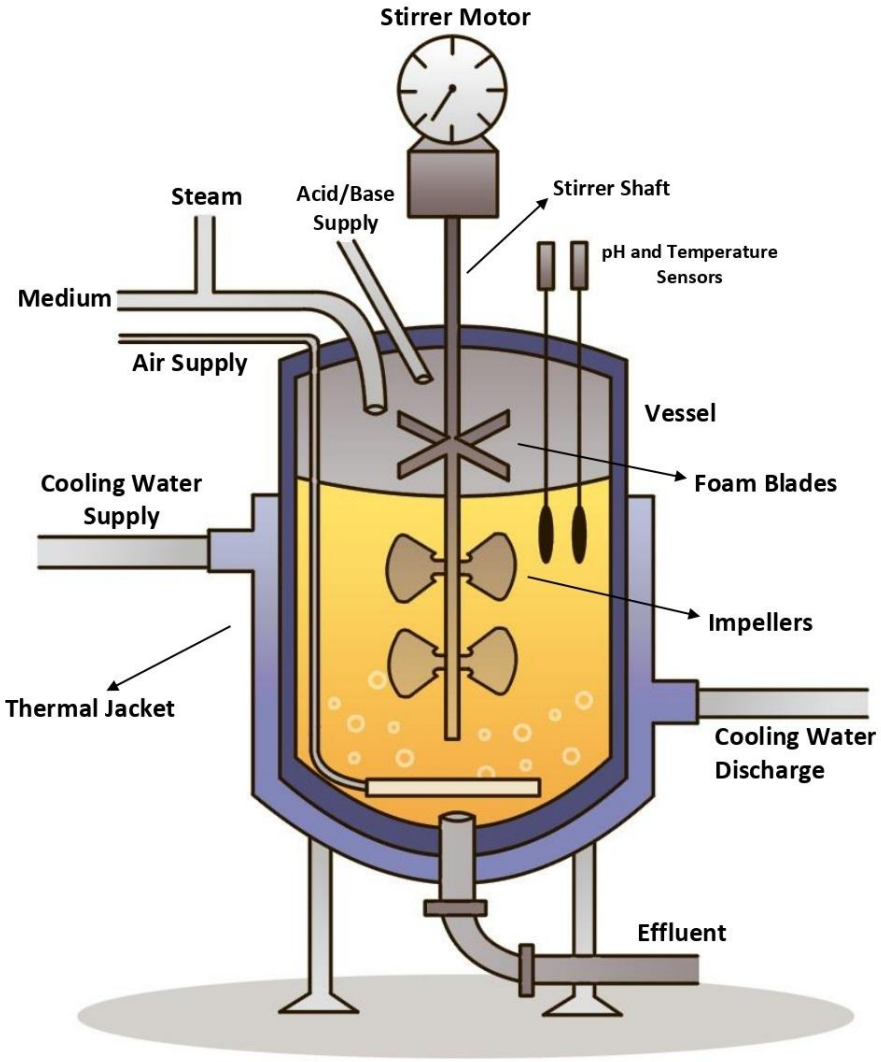
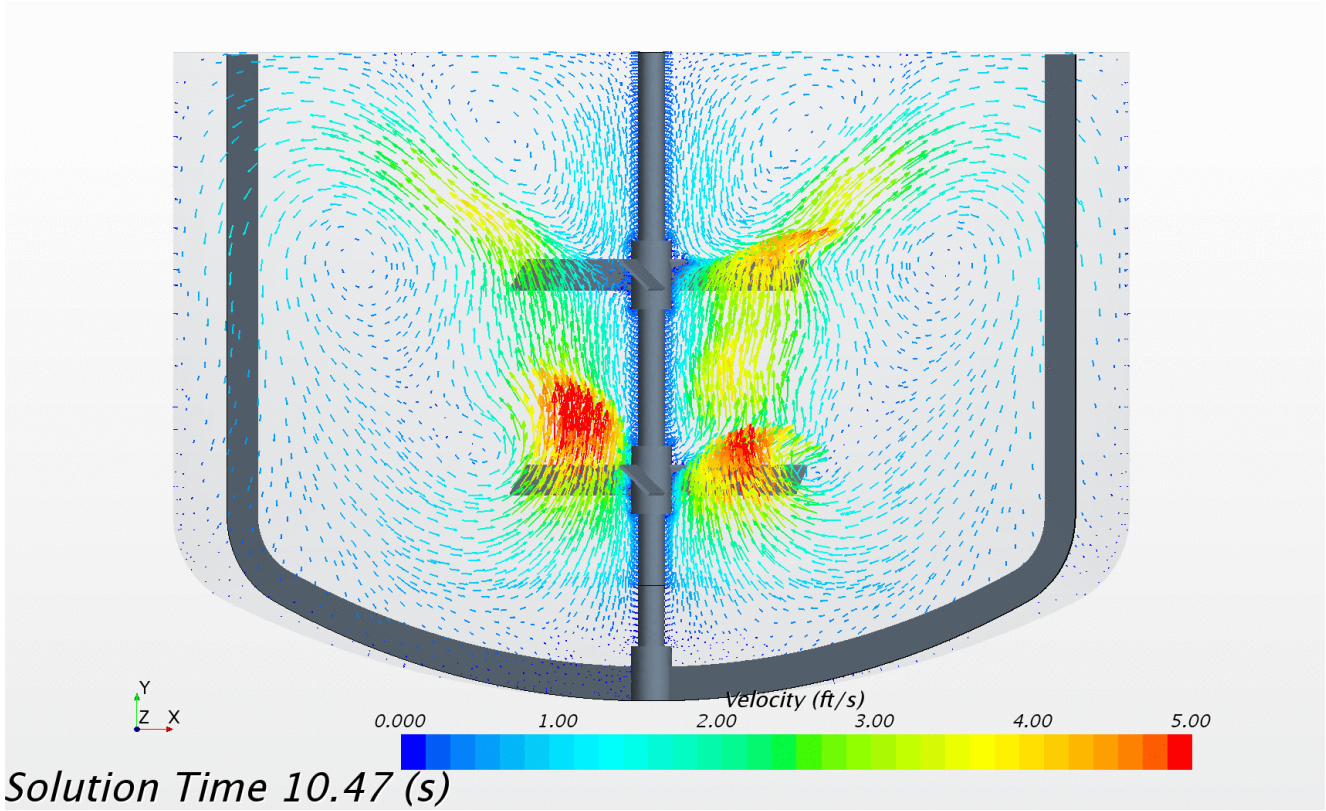
Cultivated meat manufacturing: Bioreactors





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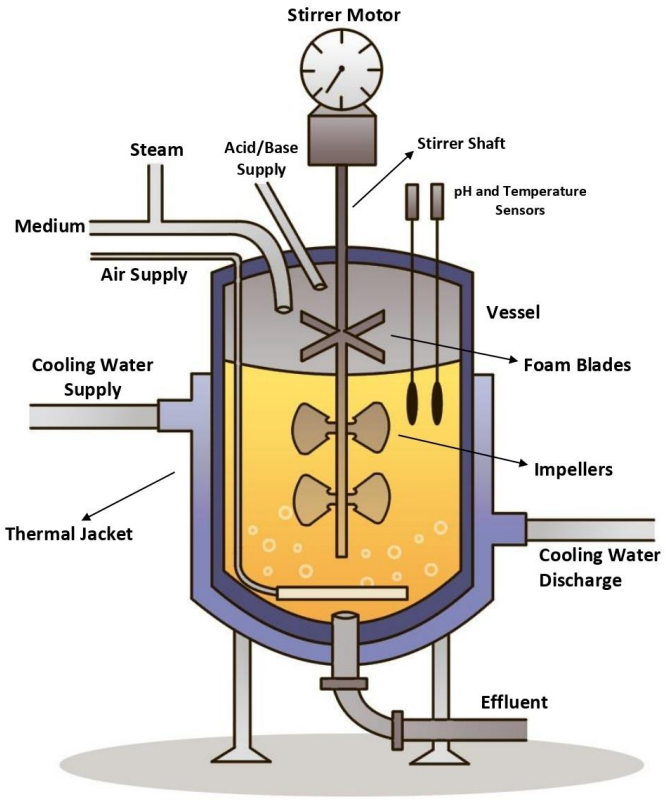
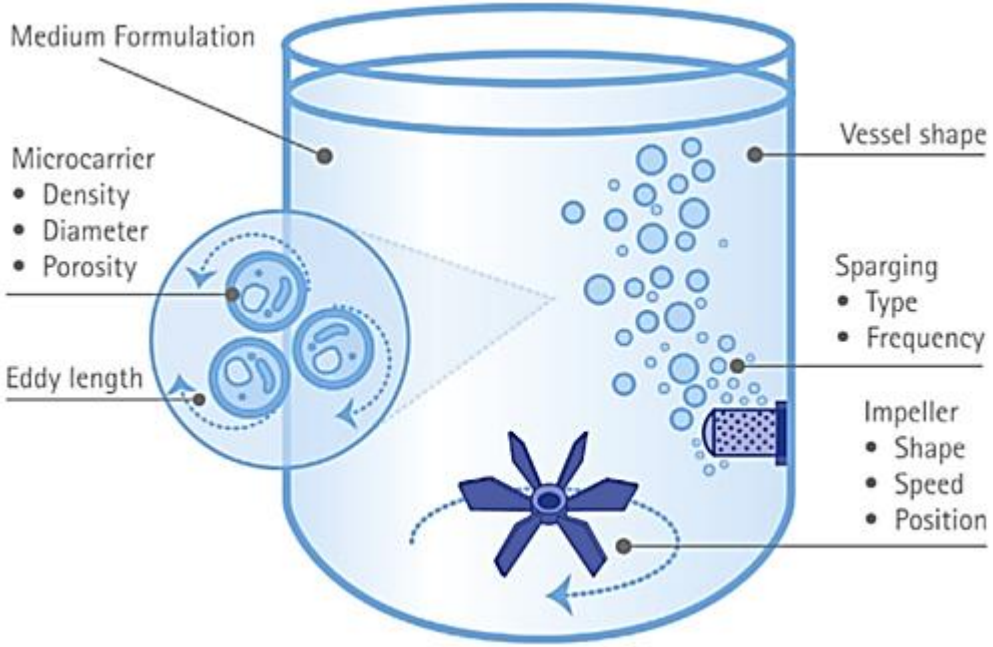
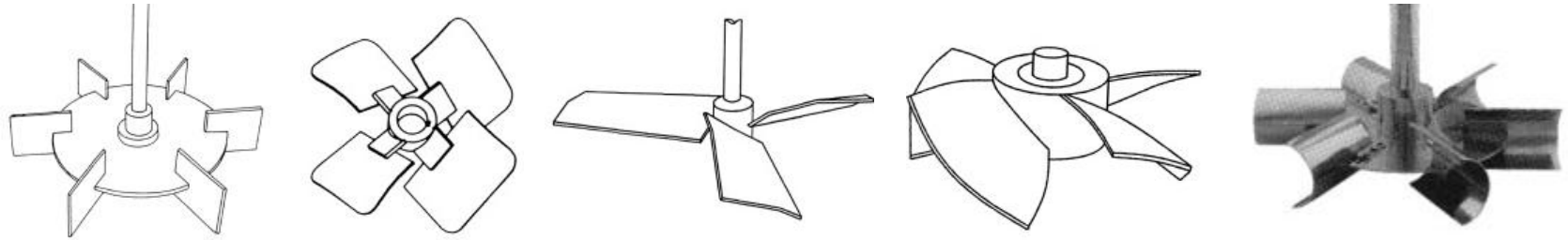
Cultivated meat manufacturing: Bioreactors





Critical factors:

- Mass transfer efficiency (O_2, CO_2, nutrients distribution)
- Shear stress on cells
- Bubble formation/rupturing
- Temperature control
- pH and metabolic waste (lactate, ammonia) management
- "Gradients"



Chisti Y, Moo-Young M. Bioreactors, Ed. Robert A. Meyers, in Encyclopedia of Physical Science and Technology (3rd Edition), 2003, Academic Press. https://doi.org/10.1016/B0-12-227410-5/00067-3.

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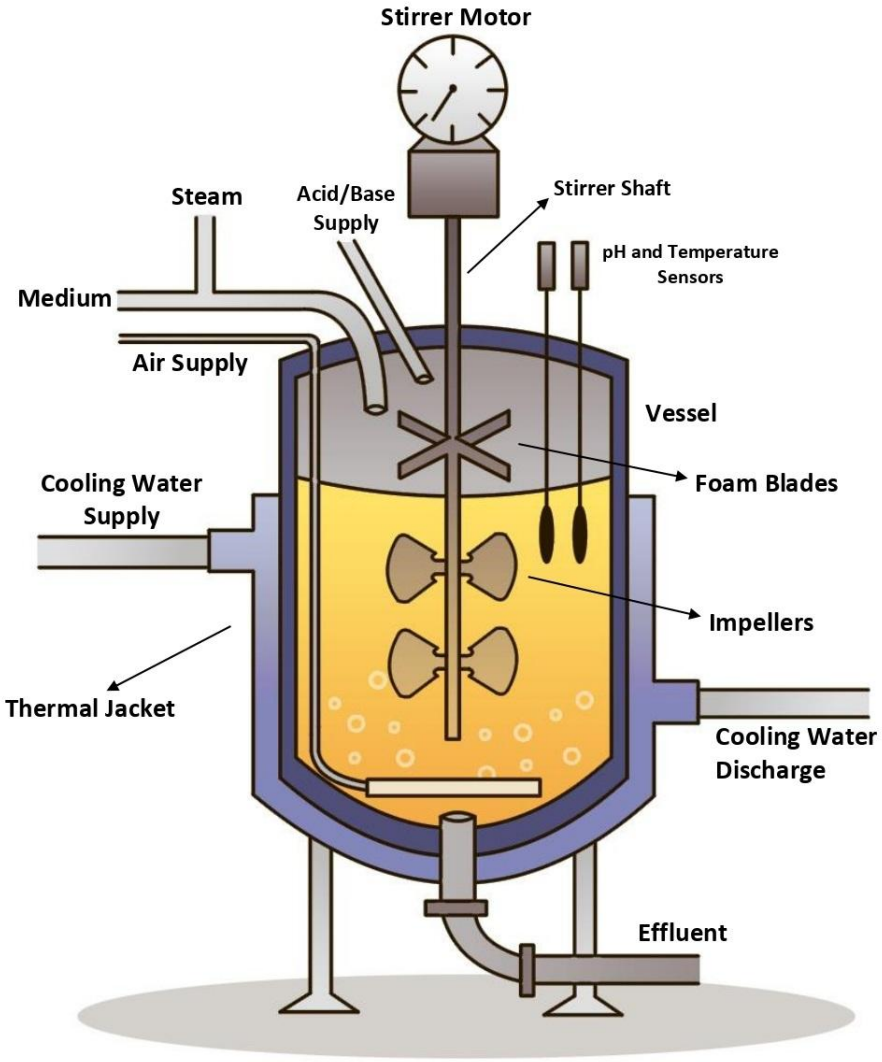
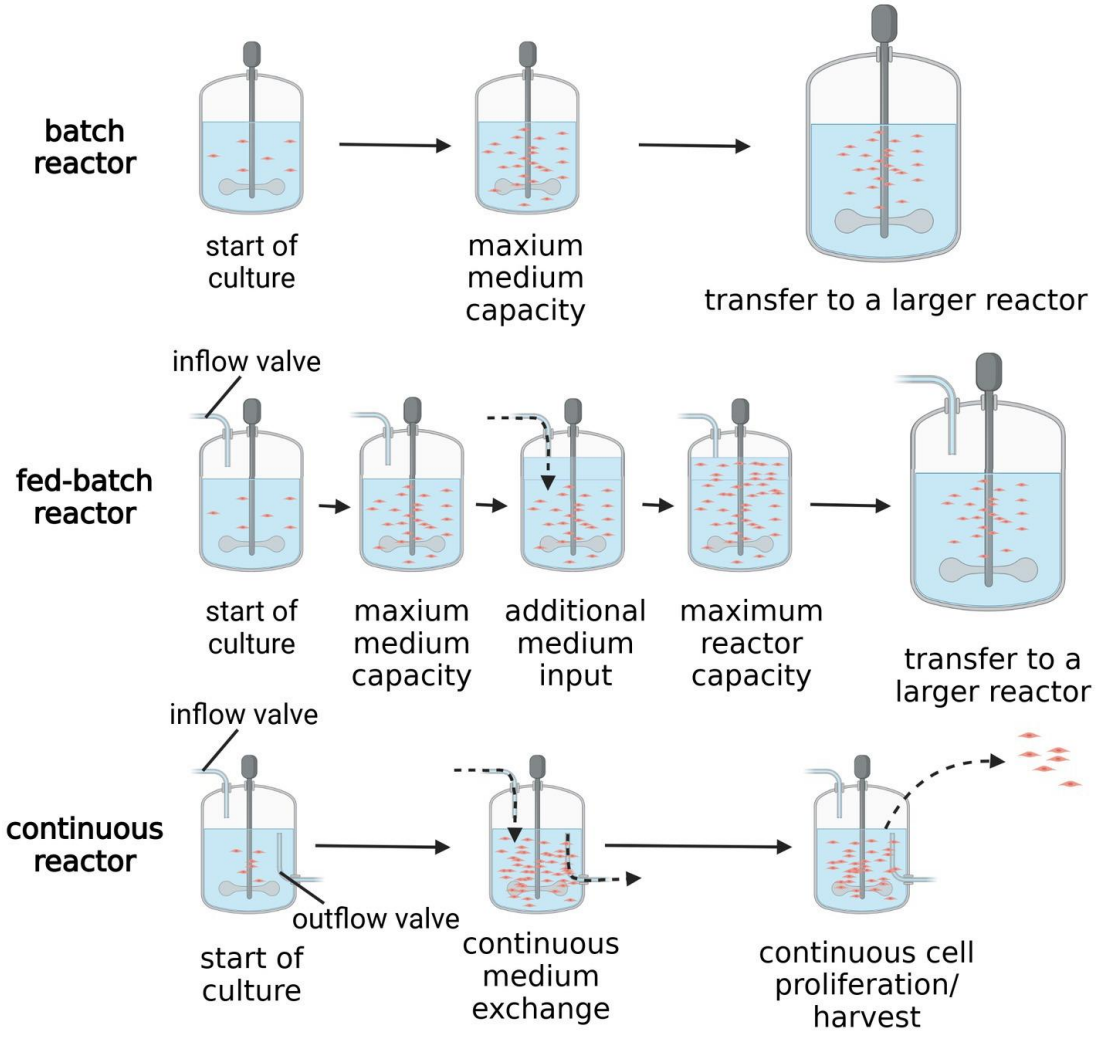
Cultivated meat manufacturing: Bioreactors





Kulus M, Jankowski M, Kranc W, et al. 2023.. Bioreactors, scaffolds and microcarriers and in vitro meat production—current obstacles and potential solutions. Frontiers in Nutrition. 10:1225233. https://doi.org/10.3389/fnut.2023.1225233

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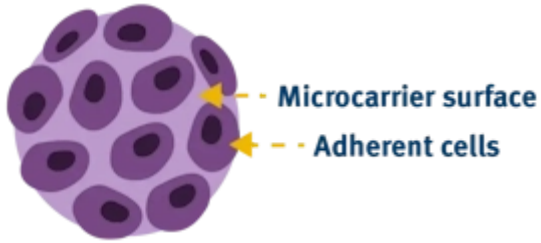
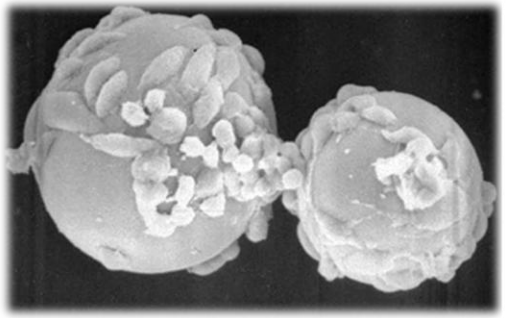
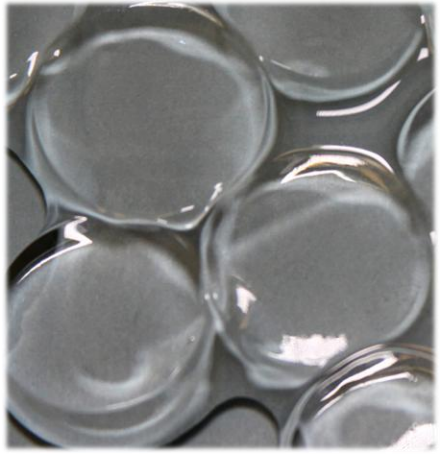
Cultivated meat manufacturing: Scaffolds

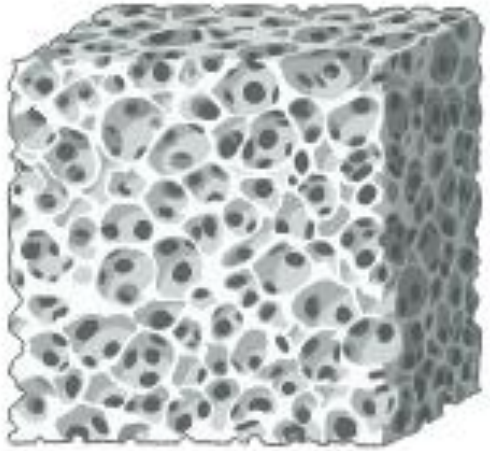




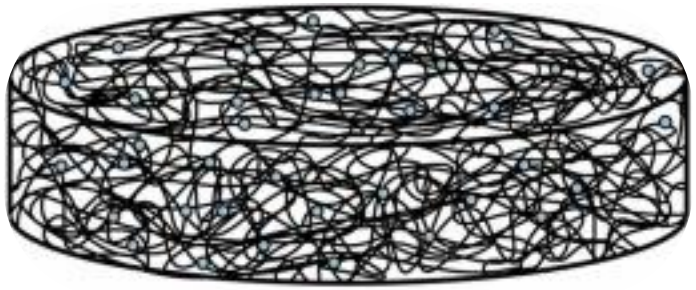
Microcarriers



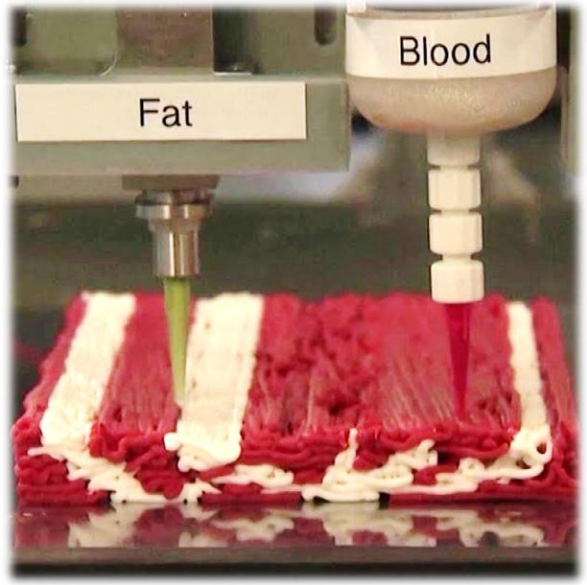
Hydrogels



Porous Scaffolds



Fiber Scaffolds
(Textile processes)



3D Printing-based

https://gfi.org/science/the-science-of-cultivated-meat/deep-dive-cultivated-meat-scaffolding/

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U.S. Proposed Regulatory Activity: Slowing Product Development?

Table 1. Cell-cultivated products that have completed regulatory review in the U.S. as of August 2025.

Year[a,b]	Product	Company
2022 FDA and 2023 USDA	Cell-cultivated chicken	Upside Foods
2023 FDA and USDA	Cell-cultivated chicken	Eat Just
2025 FDA and USDA	Cell-cultivated pork fat	Mission Barns
2025 FDA (does not need USDA approval)	Cell-cultivated salmon	Wildtype
2025 FDA (USDA approval pending)	Cell-cultivated chicken	Believer Meats

[a]Information on completion of the FDA review process from FDA Human Food Made with Cultured Animal Cells Inventory, 2025 (FDA, 2025a).

[b]Information on completion of the USDA review process from Benson & Greene (2023) and Mission Barns (2025).

Ref.: Stanley et al. (2026). Trends Food Sci., *In Press*

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Cultivated meat manufacturing: Challenges and future outlook

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- Organoleptic mimicry
- Scalability and cost reduction strategies
- Supply chain considerations
- Regulatory landscape
- Market readiness – investments and adoption

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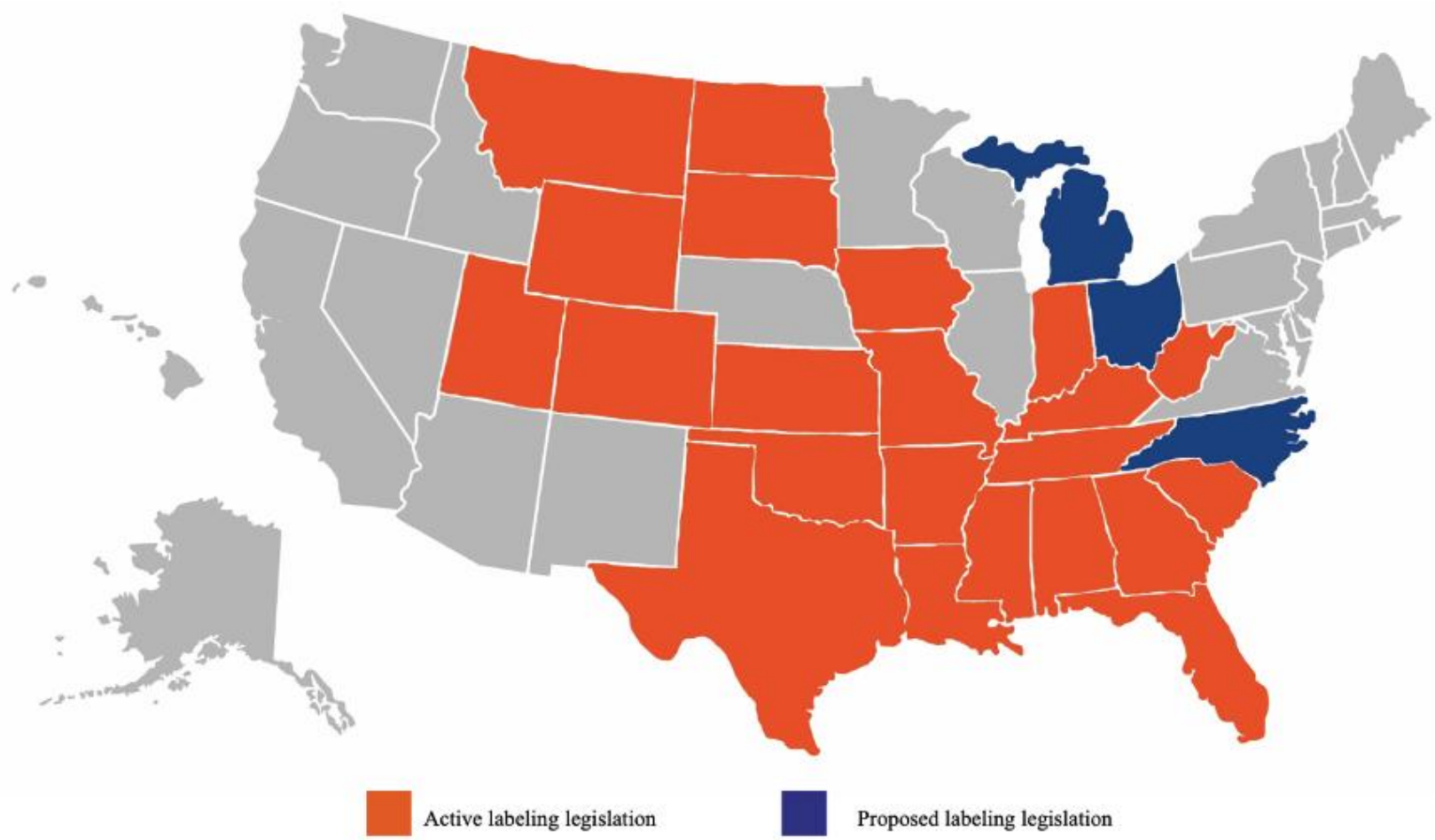
Proposed or enacted labeling legislation relevant to cell-cultivated meat and seafood products



As of August, 2025

Ref.: Stanley et al. (2026). Trends Food Sci., *In Press*

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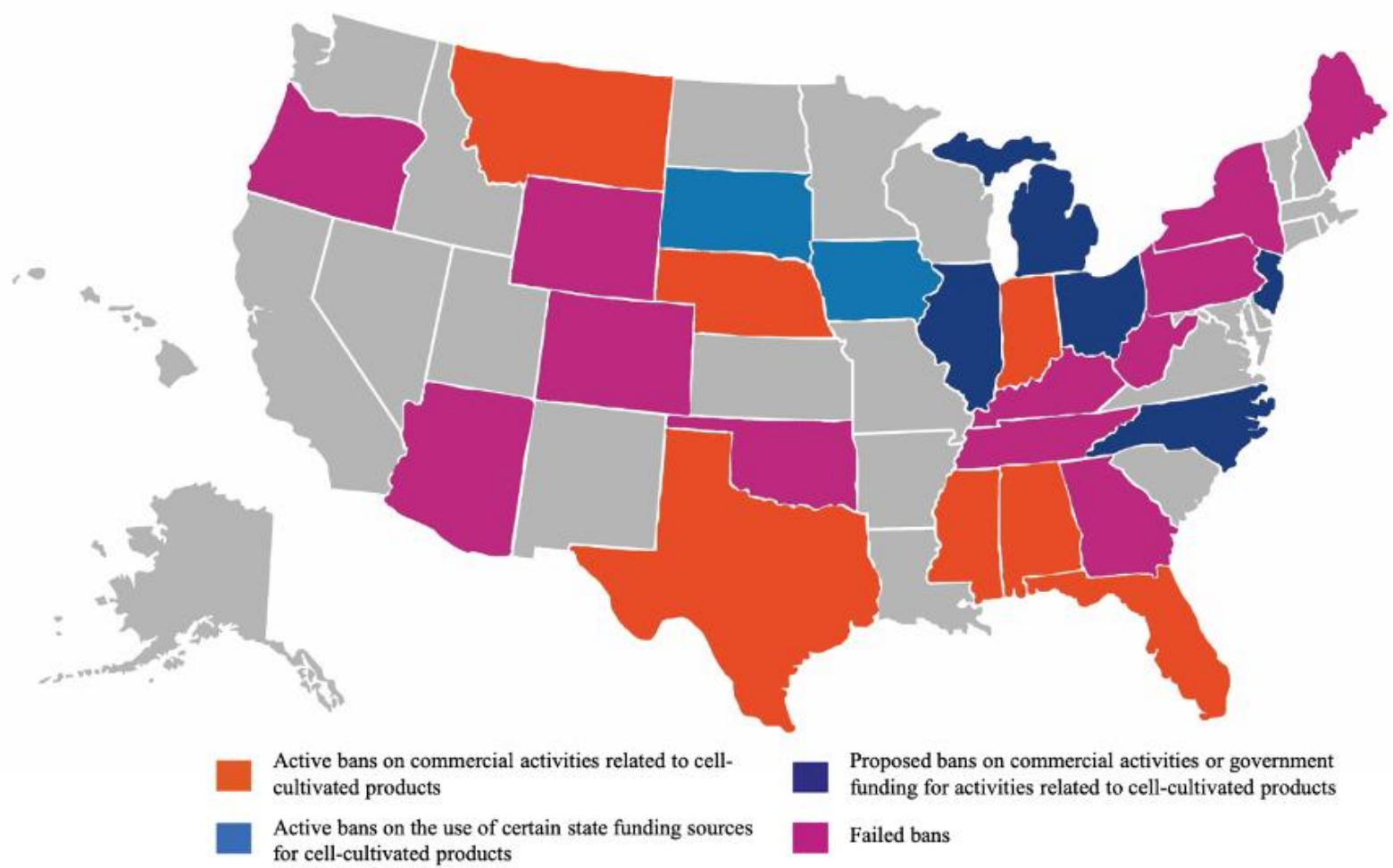
States with proposed, enacted, and failed legislation aimed to ban cell-cultivated meat and seafood production, distribution, sale, and/or research



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Ref.: Stanley et al. (2026). Trends Food Sci., *In Press*

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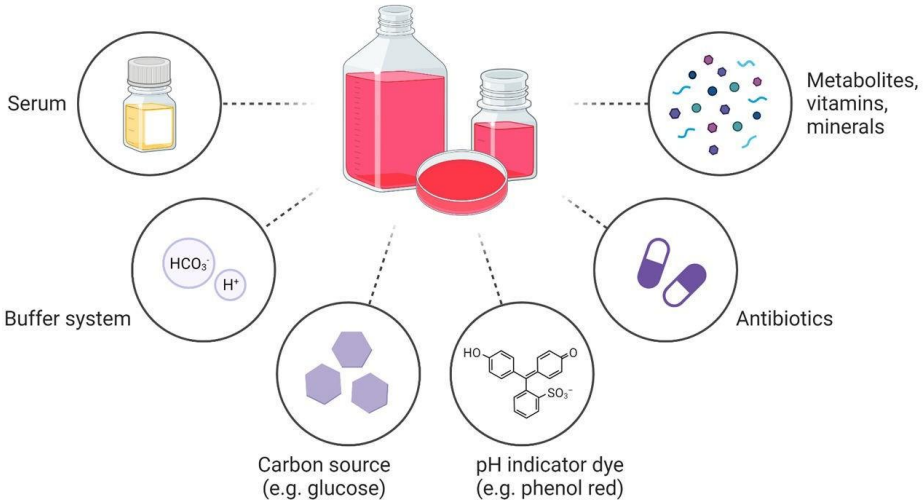
Cultivated meat manufacturing: Cell culture medium

Culture medium = Food for cells to be able to thrive *in vitro* and perform their functions



https://www.biorender.com/template/components-of-cell-culture-media

Component	Examples	Function
Basal nutrients	Amino acids, glucose, vitamins, minerals, salts	Provide essential building blocks for cell metabolism and growth
Serum/serum alternatives	A complex mixture of growth factors, hormones, amino acids, lipids etc. Fetal bovine serum (FBS) is traditionally used but chemically-defined alternatives (e.g., plant-based hydrolysates, recombinant proteins) desired for CM.	Promote robust cell proliferation and long-term cell maintenance
Growth factors (GF) and hormones	Insulin, FGF (fibroblast GF), IGF (insulin-like GF), TGF-β (transforming GF)	Regulate cell function, proliferation, and differentiation
Lipids and fatty acids	Oleic acid, linoleic acid, cholesterol	Support membrane integrity and metabolic function
Buffering agents	Sodium bicarbonate, HEPES, phenol red	Regulate pH
Antibiotics	Penicillin-streptomycin, amphotericin B	Prevent contamination

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Protein from Animal Sources

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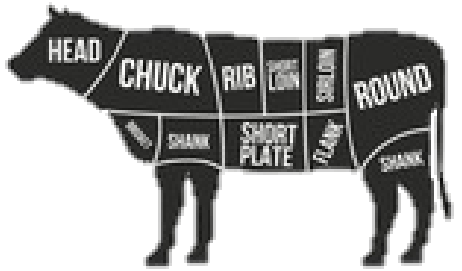
Nutritionally, why doe we consume animal products?

COMPLETE PROTEIN

(all 9 essential amino acids present)



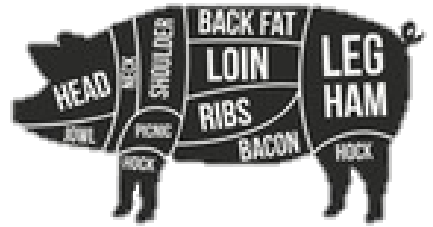


Table 2. Nutritional composition of main types of red meat (beef and pork) in USA, UK, and Spain.

	Beef			Pork		
	USA	UK	Spain	USA	UK	Spain
Energy (kcal)	126	129	131	144	124	155
Protein (g)	21.0	22.5	20.7	21.2	21.8	20.0
Fat (g)	4.0	4.3	5.4	5.9	4.0	8.3
SFA (g)	1.4	1.7	2.2	2.0	1.4	3.2
MUFA (g)	1.6	1.9	2.5	2.7	1.5	3.6
PUFA (g)	0.2	0.2	0.2	0.6	0.7	0.6
Niacin (mg)	6.2	9.7	8.1	4.8	6.9	8.7
Tiamin (mg)	0.1	0.1	0.1	1.0	1.0	0.9
Vitamin B_{12} (µg)	1.5	2.0	2.0	0.7	1.0	3.0
Iron (mg)	1.8	2.7	2.7	0.9	0.7	1.5
Zinc (mg)	3.9	4.1	3.8	2.0	2.1	2.5
Selenium (mg)	26.0	7.0	3.0	32.4	13.0	14.0
Sodium (mg)	54.0	63.0	61.0	54.0	63.0	76.0
Potasium (mg)	323.0	350.0	350.0	384.0	380.0	370.0

Data related to 100 g edible meat. SFA, MUFA, PUFA, saturated, monounsaturated, and polyunsaturated fatty acids. Modified from Delgado-Pando [40].

Macho-González A, Garcimartín A, López-Oliva ME, et al. Can Meat and Meat-Products Induce Oxidative Stress? *Antioxidants*. 2020; 9(7):638. https://doi.org/10.3390/antiox9070638

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Protein from Animal Sources

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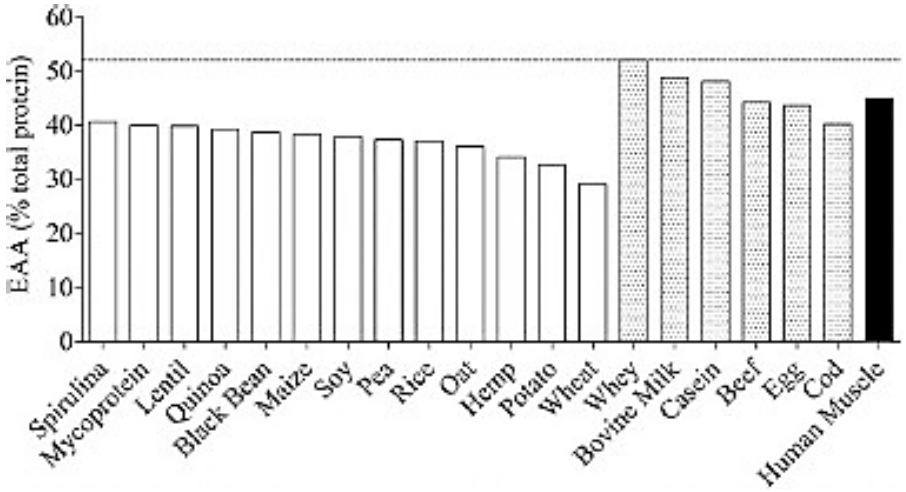

Nutritionally, why doe we consume animal products?

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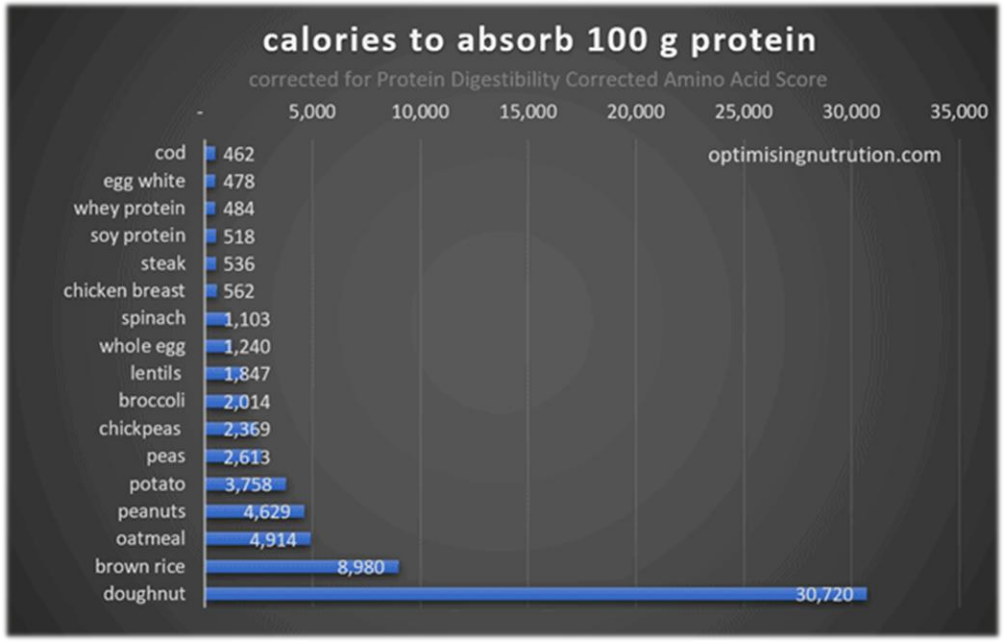

van Vliet S, Burd NA, van Loon LJC. 2015. The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption, The Journal of Nutrition, 145(9): 1981-91, https://doi.org/10.3945/jn.114.204305

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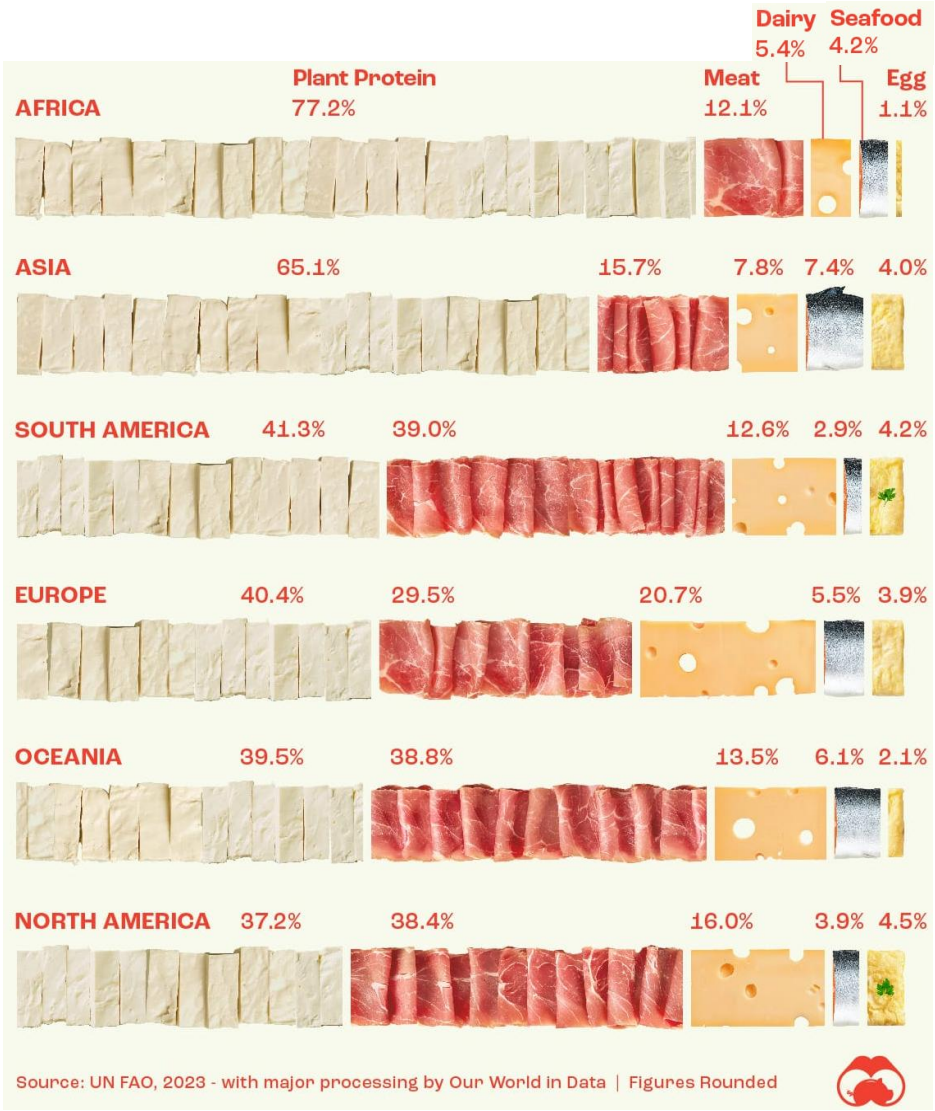
Nutritionally, why doe we consume animal products?

COMPLETE PROTEIN

(all 9 essential amino acids present)

Share of average daily protein intake (%)



KEY FACTORS:

- **Geographical**
- **Cultural**
- **Socioeconomical**

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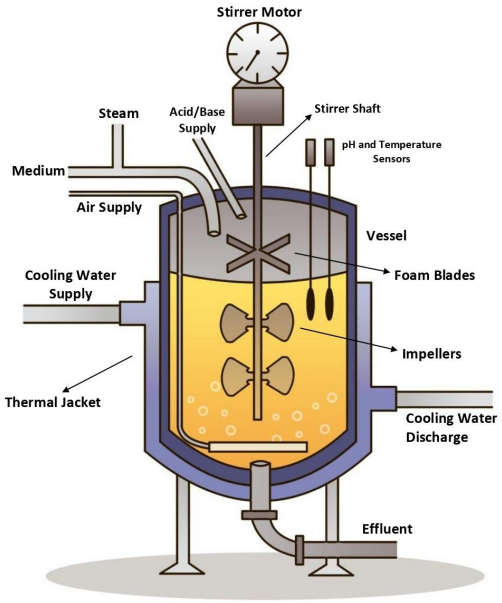
Cultivated meat manufacturing: Bioreactors



STR 50L

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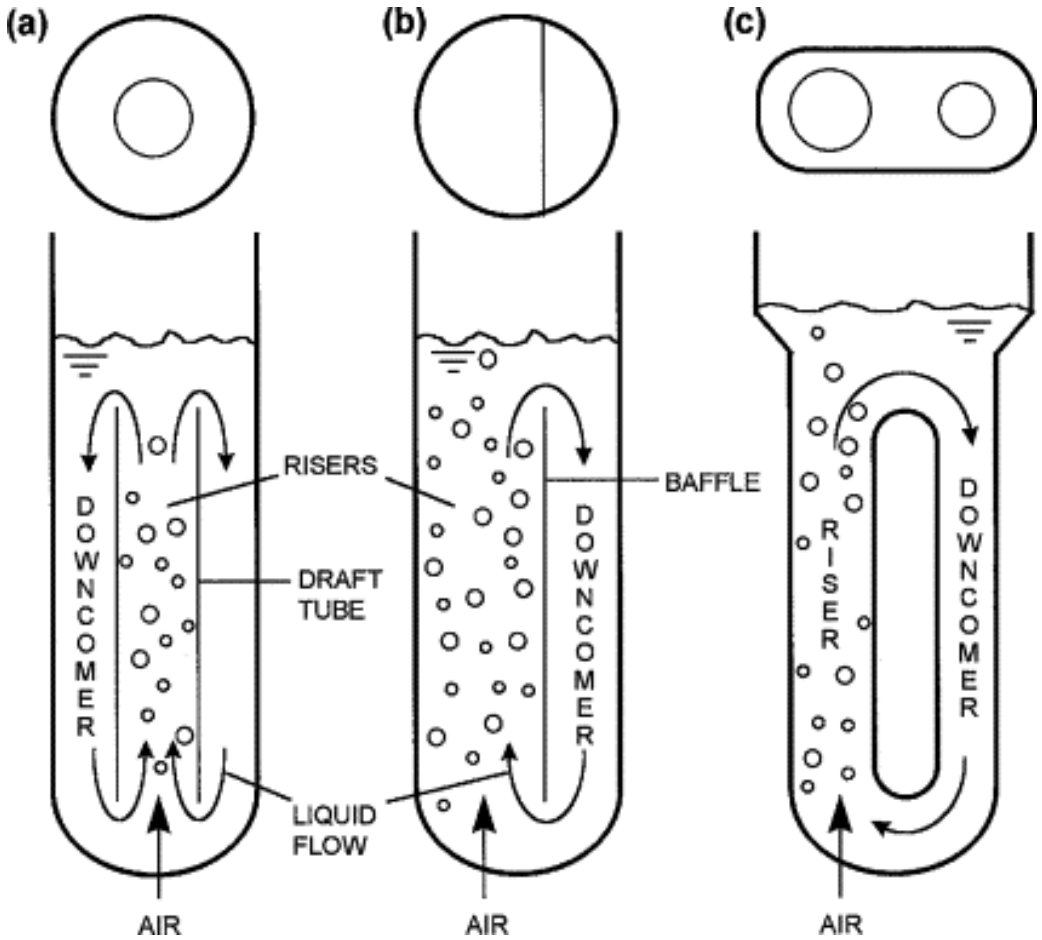
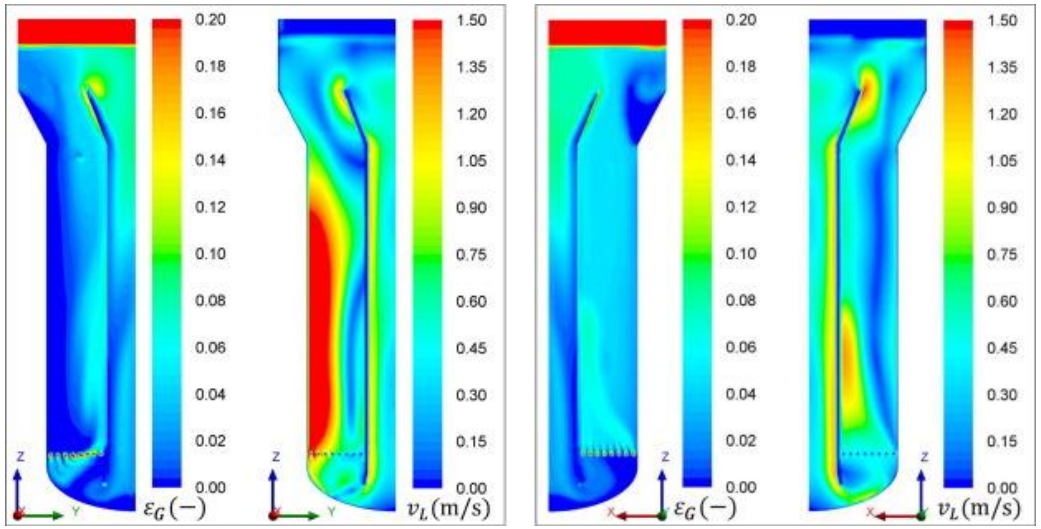
Cultivated meat manufacturing: Bioreactors





Estimate based on computational modeling:

A 300,000 L airlift bioreactor can produce enough cells to 75,000 people annually (10 kg per capita annual consumption).

Chisti Y, Moo-Young M. Bioreactors, Ed. Robert A. Meyers, in Encyclopedia of Physical Science and Technology (3rd Edition), 2003, Academic Press. https://doi.org/10.1016/B0-12-227410-5/00067-3.

Li X, Zhang G, Zhao X et al. 2020. A conceptual air-lift reactor design for large scale animal cell cultivation in the context of in vitro meat production, *Chemical Engineering Science*, 211: 115269. https://doi.org/10.1016/j.ces.2019.115269.

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Cultivated meat manufacturing: Scaffolds

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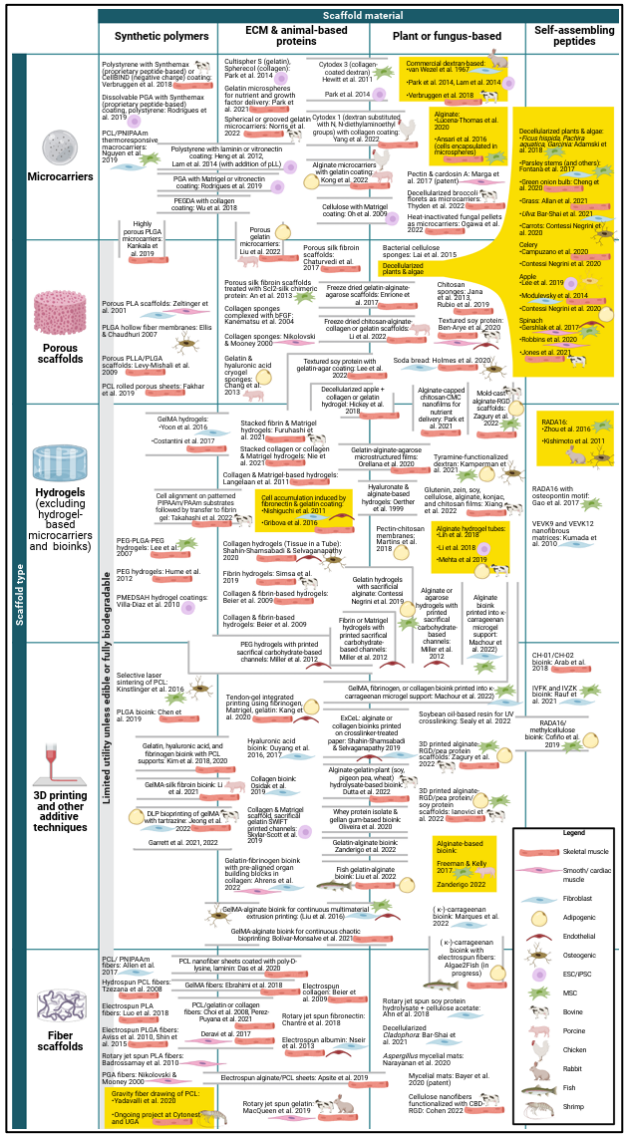

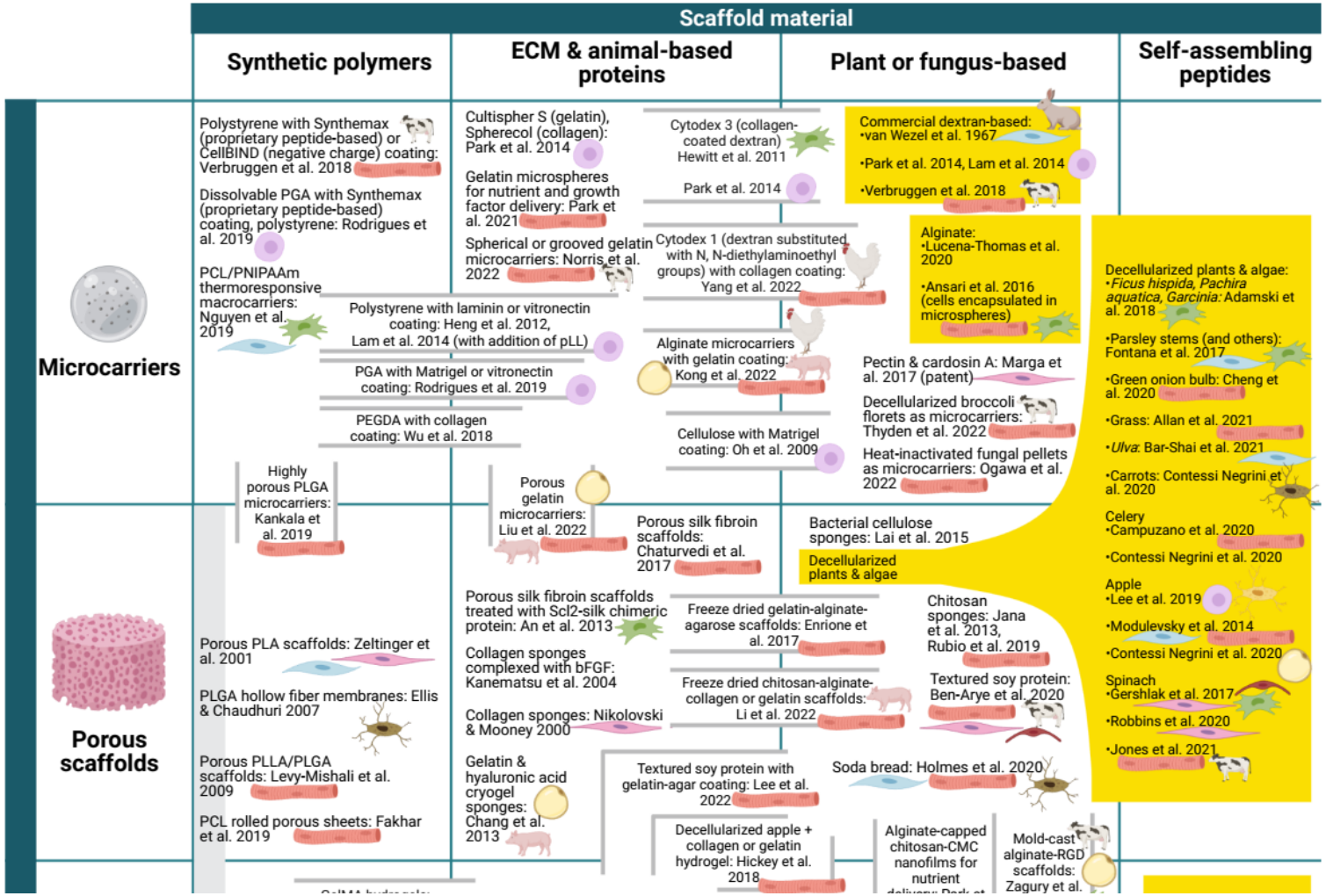

GFI Infographic: [Download] [Video]

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