Maastricht, The Netherlands

ISCCM11

Oral presentation abstracts

Opening session

You are what you eat

Luc J.C. van Loon

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Skeletal muscle protein is constantly being synthesized and broken down, with a turnover rate of about 1-2% per day. The rate of skeletal muscle protein synthesis is regulated by two main metabolic stimuli, food intake and physical activity. Food intake, or more specifically protein ingestion, directly elevates muscle protein synthesis rates. The dietary protein derived essential amino acids act as signaling molecules activating anabolic pathways and provide precursors for muscle protein synthesis. Ingestion of a meal-like amount of dietary protein elevates muscle protein synthesis rates for several hours, providing evidence that ‘you are what you just ate’. When food is ingested after a bout of physical activity the post-prandial muscle protein synthetic response is augmented, with higher muscle protein synthesis rates sustained over a more prolonged period of time. In other words, when you ingest protein following a bout of physical activity ‘you become even more of what you just ate’.

Session 1: Tissue Engineering & Biomaterials

Keynote lecture:

Prospective on structure and texture of cultivated meat and seafood: a tissue engineering approach

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The development of structured cultivated meat and seafood products may be key to foster consumers acceptance. To obtain high yields of cell biomass at scale, with high nutritional value, low environmental impacts and affordable costs, rightfully remains the dominant effort of the cultivated meat sector. Still, it is also crucial that food manufacture methodologies become available to deliver premium products with the right texture and taste.
This presentation lays the state-of-art on structuring and texturing cultivated meat and seafood with an overview of the existing manufacturing techniques. Special attention is given to sustainable tissue engineering concepts, where approaches using scaffolding, bioprinting or combination of those are used.
Results highlighting advances made at our laboratory are presented, including strategies used on fabrication of cultured fish fillet combining 3D bioprinting and electrospinning techniques. Specifically, zein-based and wheat-based nanofibers were produced and combined with 3D bioprinting of novel myogenic bioinks and fat bioinks. Plant sourced proteins, carrageenan, alginate, and methylcellulose were combined to develop suitable bioinks for the 3D bioprinting of fish cells. Finally, the 3D bioprinted cultured fish fillet organoleptic properties were enhanced by adding microalgae biomass and omega-3.

Material matters: How plant proteins shape satellite cell growth in cultivated meat

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Key challenges in cultivated meat production include finding functional, edible, and scalable biomaterials that support muscle cell growth and differentiation while also contributing to the structure, taste, and texture of the final product. To address this, we evaluated a panel of plant-derived proteins as potential scaffold and microcarrier materials, examining how their physicochemical properties influence muscle cell behaviour.

Proteins were screened for solubility, surface charge, and secondary structure to predict their interactions with cells. Bovine satellite cells were cultured on protein coatings derived from soybean, hemp seed, pumpkin seed, rubisco, peanut, chickpea, and flaxseed. We assessed the effects of each protein on cell adhesion, viability, proliferation, and myogenic differentiation. The results revealed distinct differences in how plant proteins supported muscle cell growth and fusion, with several materials promoting myotube formation and gene expression profiles consistent with enhanced myogenesis.

Our findings suggest that cell-interactive features of plant proteins—such as hydrophobicity, surface charge, and the presence of adhesion motifs—play a key role in driving muscle cell differentiation. These insights provide a framework for selecting and engineering plant-based scaffolds and microcarriers that are both biologically functional and food-compatible.

This work advances the scientific foundation for using plant proteins in muscle tissue engineering and contributes to the broader goal of making cultivated meat more viable and sustainable. Ongoing efforts will focus on scaling promising candidates and optimising them for cost-effective, large-scale production.

Mechanical optimization of edible microcarriers for improved cell differentiation and scale-up

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Edible microcarriers which can grow and direct cell differentiation are a promising method in the scale-up of adherent cell lines for cultivated meat, with the added benefit that they may be harvested alongside the final product. We have combined two FDA-cleared ingredients, combining the good cell culture performance but poor hydrogel forming abilities of one component (soya protein), with the rapid hydrogelation but poor cell attachment profile of the other (alginate). The microcarrier formulation and production process has then been stiffness-optimised via DOE to produce a series of 10 kPa scaffolds which will mechanically promote myogenic cell differentiation. The result is an edible hydrogel microcarrier with excellent biocompatibility and tuneability, which gelates rapidly in physiological conditions with minimal processing steps. We have investigated the stability of our product in standard cell culture conditions, and found that the product's mechanical stability can be ensured for up to 2 weeks via calcium addition to the media without affecting cell performance. Our most recent work is investigating the scale-up potential of these microcarriers in fluidised bed bioreactors.

Superfood-infused hydrogels for bioprinting cultured meat

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Bioprinting is a promising strategy for producing structured cultured meat, in which cells are embedded in hydrogels that act as spatial scaffolds. However, many plant- or bacteria-derived hydrogels, such as gellan gum, have little to no nutritional value, which limits their contribution to the final product. Superfoods are widely consumed for their claimed health-promoting properties and rich nutritional profiles. We therefore investigated the potential of incorporating superfood-derived extracts into hydrogels to enhance their nutritional value for use in cultured meat production.
Aqueous mucilage was produced from linseed and chia seeds, as well as an extract from avocado seeds, and was then mixed into gellan gum hydrogels. The extracts were analysed for their protein and vitamin content to confirm the presence of nutritionally relevant compounds. To evaluate the impact of the additives, we assessed the stability of the hydrogels across increasing concentrations of the extracts via dimensional and weight measurements. The weight and diameter of the modified hydrogels decreased over the 14-day culture period, but this effect was mitigated by higher extract concentrations. Furthermore, we investigated the cytotoxicity of the extracts using C2C12 myoblasts in both 2D and 3D cultures. All tested concentrations were well tolerated, indicating the biocompatibility of the additives.
In summary, linseed and chia seed mucilage, as well as avocado seed extract, show promise as natural additives for enhancing the nutritional profile of gellan gum-based hydrogels while maintaining cell viability. This approach could help to develop cultured meat products that are more nutritious and acceptable to consumers.

Optimization of porous scaffolds for muscle cell development using various fabrication methods

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This study aimed to investigate the structural characteristics of porous scaffolds for muscle cell culture, with relevance to cultured meat applications. Gelatin-based scaffolds were crosslinked with genipin and produced using four techniques: emulsion (ES), simple freezing (SS), unidirectional freezing (US), and gas foaming (GS). Each scaffold was assessed for porosity, pore size distribution, swelling capacity, and degradation behavior, alongside C2C12 myoblast responses including attachment, proliferation, and differentiation. Among the all scaffolds, ES and SS had well-connected porous structures, with ES exhibiting a smooth and uniform morphology, whereas SS displayed a torn-like and heterogeneous structure. All scaffold types exhibited swelling ratios over 1000% by 2 hours of incubation and had about 60% of pores within the 10–200 μm range, ideal for C2C12 culture. ES maintained its structural stability, showing the lowest degradation rate. Cell attachment was around 95% in ES, SS, and US. Notably, ES provided the most effective support for cell proliferation. After 14 days of differentiation, enhanced myogenic features were observed in ES, indicating favorable conditions for muscle cell maturation. These results suggest that the emulsion method offers a well-balanced combination of structural and biological advantages and is the most promising fabrication approach for skeletal muscle cell culture.

Session 2: Cell Biology & Cell Line Development

Keynote lecture:
Transcriptomic insights for satellite cell manipulation and engineering

Joshua Flack

TU Delft

Detailed understanding and accurate manipulation of cell biology is critical for efficient, cost-effective cultivated meat bioprocesses. In this keynote, Josh will explain how RNA-sequencing has given insight into the design of medium formulations, and outline future potential for the engineering of optimised cell lines.

Partial reprogramming of bovine satellite cells

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Cultured meat technology relies on the efficient expansion and differentiation of animal cells. However, no cell line is effective at both tasks; generally, cells that grow faster are slow and costly to induce differentiation, while those prone to becoming muscle or fat are hardly proliferating. This research focuses on partially reprogramming bovine satellite cells to enhance their proliferative capacity while preserving their ability to easily differentiate into muscle fibers. By introducing inducible human transcription factors (hOCT4, hSOX2, hKLF2, hMYC) via lentiviral delivery system, we aim to partially reprogram these cells without inducing full pluripotency. Our findings demonstrate that cells expressing OCT4 and SOX2 exhibited continuous growth for up to 100 days, whereas the wild-type satellite cells ceased proliferating after 10 days. RT-PCR analysis confirms successful transgene expression, with endogenous expression of bovine OCT4 and SOX2 also peaking, suggesting prolonged activation of stem-like properties. Additionally, we observed that early expression of hMYC and hKLF2 in certain conditions may contribute to initial proliferation boosts but are not mandatory. The balance between cell expansion and commitment to myogenic differentiation is critical for optimizing large-scale cell production in cultured meat applications. Our study provides insights into fine-tuning reprogramming strategies to improve bovine muscle progenitor cell efficiency, offering a novel approach to increasing biomass yield while maintaining differentiation potential. These findings contribute to advancing cellular agriculture by refining cell engineering techniques for sustainable meat production.

Fat without farms: Adipogenesis of livestock species for cultivated meat.

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Global demand for meat is expected to surge ~73 % by 2050, intensifying the environmental pressure exerted by conventional livestock farming. Cultivated meat offers a promising, lower-impact alternative, but faithfully recreating the sensory and nutritional properties of traditional meat—particularly its fat fraction, which can comprise up to 30 % of tissue mass and drives flavour, juiciness, tenderness, and caloric value—remains a critical hurdle.
Roslin Technologies has established a platform of pluripotent stem cell (PSC) lines sourced from bovine, porcine, and ovine species to address this challenge. We report a robust, scalable protocol that directs these livestock PSCs toward mature adipocytes and seamlessly transitions the entire process from adherent to suspension culture. Key advances include:

- Suspension adipogenesis: Differentiation was successfully executed in a 250 mL stirred-tank bioreactor, demonstrating compatibility with industrially relevant conditions and foreshadowing litre-scale expansion.
- Comprehensive characterisation: Pre- and post-adipogenesis phenotypes were evaluated by flow cytometry, lipid staining, and transcriptomic profiling. RNA-seq comparisons between high- and low-adipogenic PSC clones identified gene-expression signatures predictive of differentiation efficiency.
- Nutritional fidelity: Gas chromatography revealed fatty-acid profiles in bovine PSC-derived adipocytes that mirror key saturated and monounsaturated species present in conventional beef fat.

Collectively, these data establish a scalable workflow for generating authentic livestock fat from PSCs and provide molecular markers to guide cell-line selection and process optimisation. This work advances the commercial viability of whole-cut cultivated meats that deliver the taste, texture, and nutritional value consumers expect—without the environmental footprint of traditional animal agriculture.

Developing continuous cell lines for cultivated seafood: What do we know and why is it so hard?

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Cultivated seafood is at an earlier stage of development compared to cultivated meat, largely because aquatic cell culture research has historically received fewer resources compared to mammalian cells for biomedical or other non-food applications. Consequently, best practices for cell line development from aquatic animals are poorly understood. While many researchers from academia and industry are making meaningful progress and are motivated to collaborate and share data, information frequently falls through the cracks. Challenges include a publication bias toward positive rather than negative data and the omission of small but consequential details in publications. This makes it difficult to distill reproducible protocols, handling practices, and experimental techniques from the academic literature alone.

We hypothesized that the cultivated seafood research community may collectively have deeper insights and technical know-how for aquatic cell line development than typically reported in academic publications. Through a survey and a series of interviews, we gathered information to understand the obstacles to developing high-quality, continuous cell lines from fish and invertebrates. Based on these learnings, we developed a guide collating best practices and common pitfalls across a number of topics, including tissue sourcing, cell isolation, culture optimization, contamination mitigation, cell line characterization, and immortalization. Our aim is to equip researchers with real-world learnings, tried and tested methods, and practical tools for improving aquatic cell culture and cell engineering outcomes, and accelerating cultivated seafood development. We welcome feedback from aquatic cell culture practitioners as to how this guide can be improved or expanded to better deliver on that goal.

Short heat stress boosts fat formation in MSCs: Toward affordable cultured meat

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Cultivated meat, which aims to replicate traditional meat using tissue engineering and stem cell biology, is a promising approach to sustainably supplementing traditional meat production to meet increasing global demand. The production of cultivated whole-cut meat is not trivial; it requires a complex structure that supports cell growth, enables nutrient and waste exchange, and mimics natural texture. Here, we develop a biocompatible, porose, and anisotropic scaffold, based on directional freezing of nano and microcrystalline cellulose, which supports the growth and differentiation of bovine mesenchymal stem cells toward fat and muscle lineages. Furthermore, we show that preloading the scaffolds with growth factors directing the cells for proliferation or differentiation is a promising alternative to conventional media delivery since these pretreated scaffolds yield similar proliferation and differentiation efficiencies using at least 10 times lower masses of prohibitively expensive factors, and thus may significantly lower one of the primary boundaries to price parity with traditional meat. Together, these findings propose a method for the production of cultivated whole-cut meat—a sustainable and ethically preferable alternative to meet the growing demand for a highly sought-after product.

Session 3: Enabling Technologies

Keynote lecture:

Synthetic Biology: Harnessing RNA for precision post-transcriptional regulation

Fabiana Calandra

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RNA molecules can do much more than carry genetic information — they can be engineered to sense signals, process information, and control gene expression. In recent years, RNA-based circuits have emerged as a powerful framework for programming cellular behavior through post-transcriptional regulation. By designing RNA elements that interact through predictable base-pairing or protein binding, it is now possible to implement logic operations, feedback loops, and dynamic control of mRNA translation and stability. These RNA-encoded systems provide a compact, versatile, and rapidly tunable platform for constructing sophisticated genetic programs, opening new directions for synthetic biology and RNA-based therapeutic design.

Serum-free long-term expansion and functional maintenance of satellite cells via targeted genomic engineering

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Cultured meat holds great promise as a viable alternative for future meat consumption. However, the progress of research and industrialization has been limited by the lack of serum-free media capable of supporting the long-term expansion of specific cells, such as satellite cells (SCs). In this study, we developed a serum-free proliferation medium (A19), which maintained the robust cell expansion of porcine SCs at early passages. Subsequently, using CRISPR/Cas-mediated genomic editing, we established CDKN2A-/- SC lines that exhibited enhanced proliferation and differentiation capacity in serum conditions compared to CDKN2A+/+ SCs. Notably, the serum-free medium allowed for the vigorous expansion of CDKN2A-/- cells over 15 passages, while maintaining the expression of key myogenic factors PAX7, MYOD and MYOG. Further, we optimized the formula of serum-free differentiation medium, which effectively induced myogenic differentiation of serum-free cultivated CDKN2A-/- SCs. Collectively, our study establishes a comprehensive serum-free system for the entire process of cultured meat production, achieving serum-free long-term expansion and functional maintenance of SCs through targeted genomic engineering of CDKN2A gene locus.

Integrated strategies for lamb cell line development, serum-free media optimization, and culture media recycling

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Primary muscle and fat cells were isolated from 10-month-old Suffolk lambs and subsequently immortalized to overcome the replicative limitations of primary cells. Satellite cells were immortalized using hTERT/hCDK4, while fat cells employed hCDK4. Cell identities were confirmed via immunostaining and qRT-PCR: adipocytes expressed CD90 and key adipogenic markers (ACC, AFS, LEP, LPL, METTL1), while satellite cells were positive for PAX-7 and myogenic markers (ABCC4, IGF1R, PLA2G16). RNA-Seq analysis confirmed genetic stability post-immortalization, with no significant alteration in cancer-associated gene expression profiles.

To eliminate reliance on fetal bovine serum (FBS), a novel serum-free medium was developed and optimized using Central Composite Design and Response Surface Methodology. This formulation improved proliferation and viability of lamb muscle cells in both 2D and 3D cultures, achieving parity with FBS-based media in terms of cellular performance while significantly reducing cost and ethical concerns. With culture medium constituting up to 95% of cultivated meat production costs, sustainable strategies for media reuse are critical.

Recycling approaches focus on removing growth-inhibitory waste metabolites generated by cellular metabolism. Effective removal of these metabolites is essential to maintaining cell productivity and realizing economically viable, large-scale production. To address this challenge, we developed and optimized wastewater ammonia recovery techniques to remove ammonium ions from spent media.

Advancements in cell line development, serum-free media formulation, and nutrient recycling collectively establish a robust foundation for the future scalability and sustainability of cultivated meat production.

Lessons from biopharma: Reimagining mammalian cell metabolism

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Mammalian cell lines, primarily CHO cells, have been the workhorse for biotherapeutic protein production for ~40 years. Despite advances in process intensity/efficiency, universal mammalian cell phenotypes such as lactate and ammonia production, as well as the obligate requirement to supply essential amino acids, have led to challenges in process optimization without a universal solution. These same phenotypes exist—and pose challenges—for cultivated meat cell lines/processes. Over the last 9 years, we have developed genetic engineering strategies addressing these ubiquitous phenotypes in CHO cells and will present case studies for each:

Lactate: We have identified a multiplex knockout strategy that eliminates lactate production without impacting growth. This strategy is also effective in other mammalian cell lines such as HEK293.

Ammonia: Simultaneous knockout of asparaginase and glutaminases eliminates ammonia production during growth and reduces total ammonia levels over the full culture. These knockouts can be combined with the lactate knockouts, leading to cell lines with no lactate production and decreased ammonia production, again, without impacting growth.

Essential amino acids: We have restored biosynthesis for 2 essential amino acids by introducing heterologous genes from multiple microbes. The resulting heterogenous pools show minor decreases in growth, though subcloning and/or optimization around pathway gene expression may help restore normal growth. We are also working to introduce these strategies into the engineered lactate/ammonia cell lines.

While their direct translatability to cultivated meat cell lines remains to be explored, if effective, these engineering strategies have the potential to address many long-standing challenges in the field.

Engineering immortalized bMSCs with inducible MyoD for efficient muscle tissue generation in cultivated meat

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The growing demand for sustainable protein sources has intensified interest in cultivated meat as an alternative to conventional livestock farming. A major limitation in scaling up cultivated meat production is the restricted proliferative capacity and extended differentiation time of primary mesenchymal stem cells (MSCs), along with the high cost of required growth factors. To address these challenges, we developed an immortalized bovine mesenchymal stem cell (bMSC) line featuring an inducible MyoD expression system, enabling long-term expansion and controlled myogenic differentiation.

The immortalized bMSC line retains stable proliferative capacity without signs of senescence, offering a consistent and renewable cell source for muscle tissue engineering. MyoD, a key transcription factor involved in muscle development, is expressed under inducible control, allowing precise regulation of myogenesis. However, induction of MyoD alone was insufficient to initiate full myogenic differentiation. Only in the presence of myogenic differentiation media did the cells progress through the myogenic program, forming elongated, multinucleated myotubes.

Compared to wild-type bMSCs, the engineered line demonstrated significantly faster and more robust differentiation, with strong upregulation of both early and late myogenic markers observed within 10 days. The differentiated cells displayed high nuclear content and fiber-like architecture, characteristic of functional muscle tissue.

To demonstrate its application in structured meat production, the engineered cells were seeded onto cellulose-based scaffolds, which supported muscle fiber alignment and tissue organization. This inducible, immortalized bMSC platform provides a scalable, efficient, and controllable system for muscle tissue generation and represents a key advancement for cultivated meat technologies.

Session 4: Media Development

Keynote lecture:

Data-driven approaches to accelerate media design for cell-based applications

Soraya Padilla Lopategui

Multus Biotechnology

At Multus, we are advancing how cell culture media is designed across applications in cell-based biomanufacturing. Our development platform integrates high-throughput robotic automation, cell biology, and data science to accelerate the discovery of performant, cost-efficient formulations. Through systematic experimentation across thousands of media compositions, we've generated comprehensive datasets describing cellular responses under diverse conditions, including applications relevant to cellular agriculture and cultivated meat production. This data-driven approach has transformed how we interpret biological variability, enabling predictive models that guide media optimisation with increasing precision.

This presentation will demonstrate how advancements in automation, data capture and predictive modelling have shortened our development timelines, expanded our design space, and enhanced our ability to improve media performance. We will share some methodological insights from our work with CellAg-relevant cell types while highlighting how these approaches can be applied across different applications. Our research illustrates how data-driven methodologies can transform the pace and precision of media innovation, connecting fundamental research to practical applications in the industrial bioeconomy.

Sustainable food-grade serum-reducing biomaterial: Plant protein hydrolysates for scalable cultivated meat manufacturing

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Background & Objectives
Cultivated meat (CM) offers a sustainable protein alternative, but widespread commercialisation is hindered by the high cost of cell culture media (CCM) manufacturing and ethical concerns of using fetal bovine serum (FBS). In this study, we investigated bioactive plant protein hydrolysates (PPHs) as food-grade, cost-effective FBS substitute in CCM, aiming to support porcine satellite cells (PSCs) proliferation, differentiation, and CM formation on edible scaffolds.

Materials & Methods
PPHs are enzymatically derived from legumes and cereals using food-grade proteases with different substrate preferences under varied hydrolysis conditions. PPHs were characterised by degree of hydrolysis and molecular weight. PPHs were added to basal media with or without FBS, and PSCs were cultured in 2D and 3D edible plant-based scaffold. Amino acid (AA) metabolism was evaluated, cell proliferation in scaffold was evaluated using CCK8 assay. Cryosection samples were used for confocal imaging, and texture profile of the CM was assessed.

Results & Discussion
Benchmarking against standard growth media with 10% FBS, a range of PPHs enhanced the ability of basal media to maintain PSCs viability without serum. Selected PPHs supported the proliferation, maintained differentiation capabilities of PSCs under low-serum conditions, and promoted cell attachment and proliferation in 3D culture. PPHs significantly altered cellular AA metabolism, enabling enhanced cell growth. A cultivated pork prototype with 16% protein content was developed, and serum usage was reduced by up to 70%.

Conclusions
PPHs represent a scalable, sustainable solution to reduce serum dependence in CM manufacturing, maintaining cell performance while significantly lowering production costs.

Analysis of the costs, production volumes, and supply chain for amino acids used in cultivated meat manufacturing

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Techno-economic and environmental analyses of cultivated meat have identified amino acids as a key cost and environmental impact driver of production. Amino acids can be sourced from individual fermentation processes or in bulk in the form of hydrolysates and peptones from various raw materials. However, there is uncertainty as to which pathway will be most fit-for-purpose for the future cultivated meat supply chain. In this analysis, we present a production volume model that quantifies the amino acid requirements for a future cultivated meat market producing 250,000 tons annually. Using information gathered from structured interviews with leading cultivated meat companies and cell culture media and amino acid suppliers, we analyze whether current amino acid sourcing methods are sufficient to meet this demand from a cost and volume perspective, what potential bottlenecks may exist when reaching these scales, and whether alternative pathways may be needed. This work provides crucial insights into the current supply chain for amino acids used in cultivated meat, including cost profiles, regulatory considerations and constraints, the opportunities and challenges when using hydrolysates from different raw materials, and future areas of focus for research, supply chain alignment, and collaboration.

A systems biology approach to media formulation for cultivated pork cell lines

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Cultivated meat production is currently limited by high operating costs due to the use of pharmaceutical grade media components. The use of food grade components coupled to media optimization can lead to significant cost savings but is hampered by incomplete understanding of cellular metabolism in production cell lines. The pharmaceutical industry has successfully shown that models can be used to optimize media and bioprocessing designs for production in Chinese Hamster Ovaries (CHOs) and the same approach can be used for cultivated meat.
In this study, we present a modeling-based strategy to optimize cell culture media for a porcine cell line. We first developed a GEnome scale Metabolic model (GEM) and paired it with detailed experimental data on biomass composition and metabolite uptake and excretion rates for a Duroc muscle satellite cell line. The current version of the model includes 8,469 metabolites and 12,484 reactions. To improve predictions made by the model, we integrated systems biology data as constraints, creating a cell-line-specific GEM. We then used this cell line specific model to predict changes in amino acid composition that leads to improved growth. We experimentally validated these predictions and confirmed that the model optimized media led to a doubling in the growth rate after modifying just a single amino acid concentration.
This work demonstrates that genome-scale models can significantly contribute to the cultivated meat industry by enabling rational, data-driven media design.

Breaking the basal medium: Microbial extracts as a shortcut to cheaper cultivated meat

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Cultivated meat has the potential to serve as a sustainable alternative to our current traditional livestock farming, yet it has not achieved significant market penetration. The high production costs, and specifically cultivation media costs, are regarded as one of the most significant economic challenges [1]. While recent research efforts mostly focus on fetal bovine serum (FBS) replacements, techno-economic analyses suggest that FBS substitution alone is insufficient to achieve commercial viability [2–5]. Amino acids account for more than 40% of the total costs of the widely used DMEM/F-12 basal medium [2]. These amino acids are typically supplied as individual, pharmaceutical-grade, highly purified ingredients, which require around 20x more energy per gram of final product than bulk chemical production [6] . We aim to replace these amino acids by using a complex microbial extract that can supply most, if not all, amino acids at once. With our in-house medium-throughput 2D screening assay, we have examined several yeast and bacteria extracts for their ability to replace amino acids in the basal medium using immortalized bovine satellite cells (iBSCs). These extracts were produced using different lysis methods and examined for their amino acid content, degree of hydrolysis, and toxicity in the iBSC proliferation assay. Preliminary data suggest that no immediate toxic effects were observed when adding up to 100 mg/L of extract. The use of more complex, feed-grade ingredients as amino acid substitute or supplementation could drastically reduce the production cost and environmental impact of cultivated meat.

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Session 5: Bioprocessing & Scale-Up Technologies

Keynote lecture:

Building the future of meat: Scaling cultivation through bioreactor innovation

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The commercialization of cultivated meat hinges on the ability to scale animal cell culture from laboratory to industrial production while maintaining performance, cost-efficiency, and regulatory compliance. Traditional bioreactor systems, designed for biopharma applications, often fall short in meeting the unique demands of large-scale cell-based meat production — from high-density adherent cultures to stringent cost constraints.

This keynote will explore the critical engineering and biological challenges of scale-up. Drawing on real-world case studies, it will highlight novel bioreactor design strategies and platform innovations that companies in the space have been pursuing to meet production objectives.

Expansion of porcine iPSC in a 5-litre stirred tank bioreactor using fed-batch cultivation and food-safe small molecule replacements

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Roslin Technologies

The application of induced pluripotent stem cells (iPSCs) in the cultivated meat industry is gaining increasing attention due to their genetic stability, high proliferative capacity, and potential for differentiation into adipogenic or myogenic lineages. However, significant challenges remain in the development of cost-effective and scalable bioprocesses, particularly related to media costs and large-scale cultivation. In this study, high cell densities were achieved in a fed-batch suspension culture using a benchtop stirred-tank bioreactor (STR) with a maximum working volume of 5 litres using porcine iPSCs. A 250 mL Ambr® 250 modular STR system was employed for live seeding of the 5 L STR. The fed-batch process utilized two distinct culture media formulations—both incorporating food safe (Generally Recognized As Safe, GRAS) ingredients in place of pharmaceutical-grade small molecules. A seeding medium was used for initial inoculation and a feeding medium was administered daily based on cell growth dynamics. After 8 days of cultivation, cell densities exceeded 3.0×10^7 cells/mL with cell viability maintained above 90%, which resulted in over 30 g/L of wet biomass. Flow cytometry confirmed the retention of key pluripotency markers, with over 80% of cells expressing NANOG, OCT4, and SOX2. These findings underscore the feasibility of scalable, cost-effective iPSC expansion for applications in the cultivated meat sector, either for direct use in cell-based products or as a precursor to further differentiation into fat or muscle tissue.

Structured cultivated meat via 3d bio-screen printing: Process design, scale-up, and bioprocess integration

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Current 3D bioprinting technologies still lack scalability for the large-scale production of structured cultivated meat. Here, we introduce 3D-bio-screen printing (3D-B-SP) as a novel and scalable biomanufacturing process for the industrial fabrication of meat alternatives. The process enables high-resolution printing of protein isolate-rich scaffolds (>20 wt%) compatible with both 2D and 3D cell culture systems. We formulated materials that exhibited strong shear-thinning behavior (flow index = 0.27) and high viscosity (>200 Pa·s), ensuring accurate ink deposition and shape fidelity. Using the formulations, structures ranging from 0.1 to 1 mm were printed with accuracies between 81% and 95.8%. To mimic marbled meat, a printable fat material was developed and integrated into the scaffolds. Alginate was included in all formulations as a binder to enable ionic cross-linking (via $CaCl_2$) for structural stability during cell culture and food preparation (e.g., pan-frying). In 2D cultures, the marbled scaffolds supported robust myoblast growth, with high actin coverage (64%) and cellular alignment (32%) after 14 days of differentiation. In addition, myosin heavy chain staining confirmed myotube formation. For 3D cell culture applications, the marbled scaffolds were loaded with bioink and differentiated over a period of seven days. Interestingly, pre-differentiation of myoblasts in 2D before seeding led to a 7.41-fold increase in cell density in 3D compared to controls without pre-differentiation ($P = 0.00039$). Following scale-up, production rates of over 100 kg/h per printing line are expected, which demonstrates the potential of 3D-B-SP as a viable platform for large-scale structured cultured meat production.

Continuous perfusion and repeated fed-batch of porcine induced pluripotent cells to develop a stable and consistent process

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Efficient scale-up of cultivated-meat production hinges on bioprocesses that maximise cell yield, lower costs, and ensure uniform product quality. Batch and fed-batch modes are constrained by modest productivity, heavy medium consumption, and rising costs at scale. We report the first continuous-perfusion platform operated with a well characterized porcine induced pluripotent stem-cell (iPSC) line, applying the workflow through five to seven sequential production cycles to meet these challenges and advance commercial relevance.

A 30-day perfusion regimen kept cells in exponential growth, lengthening harvest windows while minimising nutrient loss and metabolite build-up. Conducted in a stirred-tank bioreactor coupled to an alternating tangential-flow (ATF) filter, the process used custom automation to hold optimal perfusion rates and enable uninterrupted biomass collection. Continuous online monitoring and data capture delivered real-time control, establishing an automation blueprint for future large-scale iPSC runs that satisfy cultivated-meat cost and sustainability targets.

In parallel, we evaluated the same porcine iPSC line across repeated fed-batch cycles to gauge impacts on expansion capacity, viability, and pluripotency. After each run, cells were dissociated and reseeded into fresh bioreactors; viability, metabolic activity, pluripotency-marker expression, and final biomass were tracked throughout. Cryopreserved vials from every cycle displayed high recovery and preserved pluripotency, confirming process stability and consistency.

Altogether, these findings validate continuous perfusion and repeated passaging for porcine iPSCs, maintaining key cell traits and delivering reliable yields. The resulting dual-mode insight provides a scalable, economical pathway for cultivated-meat producers aiming to accelerate commercialisation with a robust manufacturing platform and clear techno-economic advantages.

Plasma-alginate gel fiber toward slaughter-free cultured meat fabrication

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Cultured meat is produced by enabling myoblasts to adhere, proliferate, and differentiate on edible scaffold materials. Collagen and gelatin are commonly used edible scaffolds; however, they are derived from animal tissues and require slaughter, which contradicts the ethical motivation of cultured meat.

In this study, we propose a plasma–alginate gel as a novel edible scaffold. Plasma is a biological material containing extracellular matrix proteins such as fibronectin that promote cell adhesion. Importantly, plasma can be obtained through non-lethal blood collection, providing an ethical and sustainable alternative to collagen or gelatin. Plasma is also an edible material, and we conducted Japan's first cultured meat tasting at a university using plasma as a key edible scaffold. Alginate, a natural polysaccharide from brown algae, easily forms gels via calcium ions and can be processed into fibers using microfluidic devices. By combining plasma and alginate, we developed a cell-adhesive, edible gel suitable for myoblast culture and demonstrated that cultured meat can be constructed using edible plasma as a scaffold material.

We first demonstrated that mouse myoblasts (C2C12) adhered to and proliferated on a flat plasma–alginate gel. Using a microfluidic device, we then fabricated gel fiber that supported myogenic differentiation and myotube formation. Stacking these fibers enabled the construction of a three-dimensional cultured meat structure.

These results show the potential of plasma–alginate gels as simple, ethical, and scalable scaffolds for sustainable cultured meat production.

Session 6: Environmental & Societal Impacts

Keynote lecture:

Environmental and social implications of cultivated meat: where are we, and where must we go from here?

Nicole Tichenor Blackstone

Tufts University

Cultivated meat has attracted attention for its potential to help meet consumer demand for proteins while reducing greenhouse gas emissions, improving animal welfare, and enhancing food safety. At the same time, cultivated meat may present competition to livestock producers, workers, and their communities. In this talk, I will summarize what is known about the environmental and social sustainability of cultivated meat to date, highlight research my group is leading in both spaces, and identify critical gaps in knowledge that must be addressed to move the field forward toward a sustainable future.

Vegan and vegetarian identity and willingness to try or recommend various cultivated animal foods

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This study aimed to assess vegan and vegetarian (collectively, “veg*n”) acceptance of cultivated animal foods (CAFs), including attitudes, identity markers, willingness to try and to recommend to others. A national survey of 802 U.S. adults (315 vegans, 487 vegetarians) was conducted using a structured questionnaire comparing CAFs with conventional animal-source foods (ASFs) and plant-based foods (PBFs). Statistical analyses were pre-registered, including chi-square tests, paired t-tests, and multivariable regression.

Overall, 42.8% were willing to try CAF-meat, with vegetarians more willing than vegans. Willingness to try was also higher for cultivated dairy and eggs (55.6%) than meat, and only 9% refused to try under any condition. Culinary images were rated more favorably than petri dish images, with ice cream highest and fish lowest. Excitement also followed similar patterns, with greater enthusiasm for non-meat products. Veg*ns were significantly more likely to recommend CAFs to omnivores than to veg*ns ($p<0.001$). A stricter identity boundary was observed for vegan labels, with more rejecting a vegan vs. vegetarian label for CAF-consumers ($p=0.0005$). 517 respondents (65%) supported the use of a new label; both vegans (59%, $p=0.0011$) and vegetarians (68%, $p<0.00001$) endorsed “cegan.” Differences also emerged in perceived nutrition, ethics, and naturalness of CAFs vs. ASFs and PBFs.

Our sample of U.S. veg*ns expressed nuanced attitudes toward CAFs, with greater openness to non-meat forms and visually appealing products. These findings suggest targeted communication and product exposure may increase adoption among veg*ns, who could be a motivated audience of early adopters for sustainable food innovation.

Global developments in the regulation of cultivated meat: A Comparative study of the EU, Singapore, US and Australia and New Zealand

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Cultivated meat, that is, animal flesh grown through cell culturing techniques, also termed cellular agriculture, is positioned by proponents, developers and some governments as the future of food and the solution to the multiple environmental issues associated with intensive animal agriculture. Governments around the world are starting to regulate cultivated meat. This article provides the first comparative analysis of how the United States, the European Union, Singapore, Australia and New Zealand are regulating cultivated meat. It finds that a broadly similar approach is emerging, yet there are significant differences regarding the degree of public participation, the level of consultation between the applicant and the regulator and the role of non-scientific considerations such as environmental impacts and rural livelihoods. As part of this analysis, this article identifies and applies differing, normative interpretations regarding how cultivated meat should be regulated. These interpretative paradigms are in turn connected with diverging ideas about the role of technology and regulation in addressing environmental issues.

Guidelines for environmental life cycle assessment of cultivated meat

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Rigorously investigating the environmental impacts of cultivated meat is essential to provide reliable performance benchmarks and realistic comparisons with animal-based production systems. Environmental life cycle assessment (LCA) is globally recognized as a standardized methodology to evaluate the potential environmental impacts of products and services. To date, cultivated meat LCAs have focusing on diverse products and employed a wide range of modeling choices, data quality, and assumptions. This leads to uncertainty about cultivated meat systems' impacts. Due to the nascency of the industry, LCA practitioners encounter numerous barriers in developing cultivated meat LCAs - from choosing appropriate data to modelling complex systems across scales, time, and space. To address these gaps, we assembled a global team of 25 recognized and active scientists in cultivated meat LCA, livestock systems LCA, and the International Standards Organization (ISO) LCA standards to develop guidelines for cultivated meat LCA. We used a workshop (in person and online), online meetings, and asynchronous rounds of feedback to reach consensus on guidance over the course of a year. In this presentation, we unveil these guidelines for the first time, highlighting considerations for study design, data access, and future research that are of broad interest to the cultivated meat research community. The guidelines provide specifications throughout the four phases of LCA, from goal definition to the interpretation of results. We invite practitioners to apply these guidelines when investigating cultivated meat systems to increase the consistency and reliability of environmental impact evaluations for these emerging products.

Poster session abstracts:

Poster Session A

Abstract # 1

Growth factors-infused cellulose scaffolds support cost-efficient proliferation and differentiation of bovine stem cells for cultivated meat

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Cultivated meat, which aims to replicate traditional meat using tissue engineering and stem cell biology, is a promising approach to sustainably supplementing traditional meat production to meet increasing global demand. The production of cultivated whole-cut meat is not trivial; it requires a complex structure that supports cell growth, enables nutrient and waste exchange, and mimics natural texture. Here, we develop a biocompatible, porose, and anisotropic scaffold, based on directional freezing of nano and microcrystalline cellulose, which supports the growth and differentiation of bovine mesenchymal stem cells toward fat and muscle lineages. Furthermore, we show that preloading the scaffolds with growth factors directing the cells for proliferation or differentiation is a promising alternative to conventional media delivery since these pretreated scaffolds yield similar proliferation and differentiation efficiencies using at least 10 times lower masses of prohibitively expensive factors, and thus may significantly lower one of the primary boundaries to price parity with traditional meat. Together, these findings propose a method for the production of cultivated whole-cut meat—a sustainable and ethically preferable alternative to meet the growing demand for a highly sought-after product.

Abstract # 4

from bovine stem cells to embryo models

Shadi Tawil

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Stem cells, particularly embryonic stem cells, have emerged as powerful tools for studying early mammalian development and hold immense potential in basic science and biotechnology. Gastruloids, three-dimensional aggregates of embryonic stem cells, exemplify this potential by recapitulating key aspects of embryogenesis, including symmetry breaking, axial elongation, and germ layer specification. These structures offer unique advantages over traditional embryo research, such as scalability, accessibility, and ethical considerations. Gastruloids provide insights into fundamental developmental processes, gene regulatory networks, and metabolic pathways crucial for embryonic patterning and morphogenesis. They serve as versatile models for investigating various aspects of gastrulation, somitogenesis, and early organogenesis, enabling high-throughput studies and genetic manipulations that are challenging in vivo. The applications of stem cell research extend beyond basic science to biotechnology, including the emerging field of cultivated meat. By leveraging insights from gastruloid studies on mesodermal differentiation and tissue organization, researchers can optimize protocols for generating muscle and fat tissues from stem cells for lab-grown meat production. This approach addresses key challenges in cultivated meat technology, such as scalability and tissue complexity, while offering a sustainable and ethical alternative to conventional meat production.

Abstract # 19

Generation, Characterisation, and Scale-up of Bovine Mesodermal Cells (MP and MSCs) for Cultivated Meat

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Cultivated meat must include both fat (adipocytes) and muscle (myocytes) to reproduce the nutrition, flavour, and texture of conventional meat. These tissues arise from the mesodermal lineage, yet primary mesodermal cells from livestock are scarce and poorly characterised. Pluripotent stem cells (PSCs) therefore provide a renewable route to generate the required lineages in vitro.
We established a streamlined protocol that differentiates multiple bovine embryonic stem-cell (ESC) lines into mesodermal progenitors (MPs) and, subsequently, induced mesenchymal stem cells (iMSCs). Bovine MPs display a distinctive immunophenotype, are multipotent, and give rise to downstream mesodermal derivatives including iMSCs. The resulting mesodermal cell banks have been characterised, scaled in suspension culture, differentiated into myocytes and adipocytes, and cryopreserved at high density.
ESC-derived iMSCs were further expanded in stirred-tank bioreactors and shown to differentiate efficiently into adipocytes and fibroblasts under both adherent and suspension conditions. Compared with primary MSCs, these iMSCs exhibit an extended lifespan in culture, robust and efficient differentiation capacity, and batch-to-batch consistency.
Flow cytometry and RNA-sequencing confirm that both MPs and iMSCs retain key markers of mesodermal identity and closely resemble corresponding primary cells. Collectively, our data demonstrate that bovine ESC-derived iMSCs provide a fully traceable, high-quality, and scalable cell source for incorporating muscle and fat components into cultivated beef products, addressing a central need of the cultivated meat industry.

Abstract # 21

Ovine pluripotent stem cells for cultivated lamb production: the potential of induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs)

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Global demand for protein is rising, and cultivated meat offers a sustainable alternative that eliminates animal rearing and lowers the environmental impact of conventional meat production. Successful commercialisation hinges on cell lines that are robust, safe, genetically stable and readily scalable, while retaining the capacity to differentiate into muscle and fat.
Roslin Technologies has developed high-quality ovine pluripotent stem cell (PSC) platforms—both induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs)—alongside dedicated media and protocols optimised for cultivated-meat manufacturing. PSCs are uniquely advantageous because they self-renew indefinitely yet remain capable of forming any somatic lineage.
This study outlines the derivation workflow for ovine iPSCs and ESCs and systematically compares their performance. We demonstrate maintenance of pluripotency marker expression, expansion in suspension culture, differentiation potential toward myogenic and adipogenic lineages, and genetic stability during extended passaging.
We further illustrate the adaptability of these lines through targeted gene-editing approaches that enhance muscle and fat differentiation outcomes.
Taken together, these results demonstrate that Roslin Technologies' ovine PSC lines meet the critical criteria—safety, stability, scalability and directed differentiation—required for large-scale cultivated-meat production.

Abstract # 23

Characterization of satellite cell subpopulations focusing on Notch pathway-associated markers through FACS – potential for cultivated meat

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Satellite cells have gained attention in recent years driven by the increasing interest in cultivated meat. Despite this, the use of satellite cells for large-scale production of cultivated meat is challenged by their limited proliferation capacity over time. However, the true potential of these cells is still undefined. Moreover, satellite cells have diverse stemness and myogenic capacity, due to their heterogeneity, and are regulated by several signaling pathways. The Notch pathway is known to be involved in satellite cell proliferation and self-renewal. Therefore, characterizing the subpopulations of satellite cells through transmembrane proteins and ligands involved in the Notch pathways gives insights on evolution, stemness, and proliferation capacity of the heterogeneous satellite cell pool. In this study, we aim to detect and sort subpopulations of satellite cells through Notch 1, Notch 2, Notch 3, and Delta 1 ligand antibodies using FACS. Moreover, we analyzed how different media, with or without serum, can affect the proportion of satellite cell subpopulations. Cell characterization was furthermore assessed at different time points during proliferation using immunofluorescence staining. As a result, satellite cell subpopulations influenced the proliferation capacity and the expression of myogenic markers of the cells.

Abstract # 24

Development of enhanced differentiation efficiency of bovine muscle stem cells using KnockOut Serum Replacement and small molecule screening

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Efficient differentiation of muscle stem cells into muscle fibers is required for cultured meat production. However, there are still hurdles due to undifferentiated cells, known as ‘reserved cells’ during differentiation. It is important to reduce or eliminate the reserved cells for efficient differentiation. KnockOut Serum Replacement(KSR) is an FBS-free formulation widely used in embryonic stem cell and induced pluripotent stem cell (iPSC) cultures. To date, no studies have investigated the differentiation of bovine muscle stem cells using KSR. In this study, we analyzed the effects of KSR as a replacement for fetal bovine serum(FBS) or horse serum(HS) in the differentiation of bovine muscle stem cells and optimized its concentration for efficient differentiation. We then screened 105 small molecules to identify synergistic effects on differentiation in combination with KSR. Our results showed that 3% KSR induced more effective myotube formation than FBS or HS, with a significantly higher fusion index. Small molecule screening revealed that 3% KSR combined with PD0325901(PD), Forskolin(FSK), and Urolithin B(UB) led to more efficient myotube formation than 3% KSR alone. The population of reserved cells significantly decreased with PD and UB treatments, while FSK resulted in the highest proportion of reserved cells. Fusion index analysis showed that the combination of PD and UB produced the highest fusion index compared to 3% KSR alone. Our results demonstrated that 3% KSR effectively promoted the differentiation of bovine muscle stem cells into myotubes. Furthermore, 3% KSR with PD or UB enhanced differentiation and significantly reduced the population of reserved cells.

Abstract # 26

Crusting-fabricated soy-based scaffolds yield three-dimensional muscle tissues for cultured chicken meat production

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Crusting fabrication is a non-chemical, non-machine intensive method of fabricating edible soy-based scaffolds. Pure soy homogenate (Y2R)- and soy homogenate with 1% vegetable glycerol (Y2G)-based scaffolds were developed to produce edible scaffolds for cultured meat production. Spontaneous folding of scaffolds during incubation suggests high biocompatibility for tissue development. Both Y2R and Y2G exhibited surface structures that potentially provide adhesion points for myogenic cells supported by their non-significant difference in cell seeding efficiency. However, Y2G generated superior results in terms of collagen content and tissue yield compared to Y2R. The expression of relevant myogenic biomarkers revealed non-significant differences in desmin and myogenin, albeit relatively high myoblast determination protein 1 expression in Y2R ($p<0.05$). This suggests active differentiation even after 21 d of total incubation. Y2G allowed a greater accumulation of collagen and overall cell mass, confirming the advantageousness of a smaller pore size to three-dimensional tissue formation. In terms of cultivated chicken meat production, both Y2R and Y2G proved useful as edible scaffolds, with greater preference for Y2R, considering the absence of chemical additives during its fabrication process.

Abstract # 29

Solving for Scale: Edible Scaffolds That Meet the Demands of Cultivated Meat Production

Heidi Coia

Nexture Bio

Nexture Bio develops animal-component-free (ACF) microcarriers and scaffolds for food and life science industries. Innovative 3D formats of edible materials provide the optimal environment for adherent cells to grow in a way that mimics native tissue. This enables the building of biomass, while driving organoleptic qualities, nutritional value, and the economic viability needed for cultivated meat products. Our technology incorporates techniques adopted from materials science and tissue engineering to optimize cellular growth, proliferation, and infiltration into the scaffold while demonstrating the key cellular functions and protein expression to be as similar to meat tissue as possible.

Scaffolds and microcarriers with a range of textures, shapes, sizes, formats, and plant-based ingredients are necessary to provide solutions for the variety of cell, bioreactor, media, and downstream production methods currently under investigation in the cultivated meat industry. Although to date the industry has yet to provide commercial production of ACF microcarriers and scaffolds that are safe and suitable for use in food, Nexture Bio has the cellular performance, material composition, and data to demonstrate our ability to scale to meet the demands of the industry.

We will demonstrate how our cell growth metrics align with familiar off-the-shelf products used for 3D cell culture growth in other life science industries. In addition, we have evaluated our leading materials with cell culture in challenging conditions, using FBS-alternative media and suspension environments. These achievements suggest that cultivated meat producers can use ACF materials in novel formats in their processes to achieve high cellular biomass production.

Abstract # 30

Cell cycle arrest induced by MEK inhibition promotes porcine myogenic differentiation

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Cultured meat has emerged as a viable, sustainable protein source that can advance environmental conservation and enhance animal welfare. However, several challenges must be addressed for its successful industrialization, and one of the key limitations is insufficient muscle differentiation in vitro. This limitation hampers the replication of conventional meat and reduces production efficiency. To address this, we explored the modulation of signaling pathways involved in myogenesis. Among them, we specifically investigated the MEK pathway, which is activated during the proliferation phase, and assessed the impact of its inhibition on the differentiation of pig muscle stem cells (pMuSCs). As a result, MEKi treatment accelerated and elevated MyoG, MYH4, and MYH7 expression. Consistently, immunofluorescence staining for MYH demonstrated a significant increase in the extent and speed of myotube formation. Mechanistic analysis revealed that, compared to the untreated group, MEKi treatment induced cell cycle arrest from the early stages of differentiation via upregulation of p21 and p27 and suppression of Cyclin D1/CDK4 which rapidly drove the transition to differentiation. Furthermore, we evaluated whether enhanced differentiation would influence flavor-related components by analyzing free amino acid and nucleotide profiles. However, despite the improved differentiation, no significant changes were observed in these flavor precursors. These findings highlight the need for further studies employing long-term culture systems capable of generating mature muscle tissue. Collectively, our results suggest that MEK pathway inhibition is a promising strategy for enhancing muscle differentiation in vitro and may contribute to improving the structural and production efficiency of cultured meat.

Abstract # 32

Application of an MSGN1 Reporter System in Skeletal Muscle Differentiation from Pig Embryonic Stem Cells for Cultivated Meat Research

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Skeletal muscle differentiation using pluripotent stem cells (PSCs) can provide a powerful platform to produce cultivated meat without animal sacrifice due to their self-renewal ability. MSGN1 has been recognized as a core factor for the paraxial mesoderm lineage, which is the earliest mesoderm in the developmental process of skeletal muscle. Accordingly, the MSGN1-based reporter system for tracing the paraxial mesoderm offers a useful tool for monitoring the induction of mesodermal lineage and cell fate transition during the differentiation into the myogenic lineage from PSCs. Therefore, we established a pig embryonic stem cell line (ES cell line) carrying a porcine MSGN1 upstream-based reporter system. The integration of the reporter system was confirmed by genotyping PCR and sequencing. The engineered pig ES cell line maintained the pluripotency as indicated by alkaline phosphatase activity and the expression of pluripotent markers. Especially, the co-expression of SSEA1 and SSEA4 was observed, demonstrating the retention of porcine species-specific features of pluripotency. The differentiation capacity into trilineage was evaluated through spontaneous differentiation and directed differentiation. Under differentiation conditions for paraxial mesoderm, the reporter activity was detected and simultaneously, MSGN1 was upregulated in the FACS-sorted EGFP+ cells, verifying the functionality of the reporter system. The MSGN1 reporter cell line was stably maintained during an extended period without chromosomal mutation. Therefore, our system can contribute to cultivated meat production by analyzing the paraxial mesoderm-mediated myogenic differentiation process and ultimately developing tailored culture conditions with high differentiation efficiency.

Abstract # 34

Curcumin Enhances Differentiation of Hanwoo Muscle Satellite Cells for Cultured Meat Applications

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This study investigated the effects of curcumin supplementation on the myogenic differentiation of satellite cells isolated from Hanwoo (Korean native cattle) muscle to enhance muscle cell differentiation capacity for cultured meat development. Primary muscle cells were harvested using enzymatic dissociation from the semimembranosus muscle and further purified by fluorescence-activated cell sorting (FACS) to isolate $CD31^-$, $CD45^-$, $CD29^+$, and $CD56^+$ Hanwoo satellite cells. To evaluate the role of curcumin, cells were cultured in differentiation media containing 1% DMSO (control), DAPT (10 nM, 100 nM, 1 μM), or curcumin (1, 5, 10, 50, and 100 μM). Morphological changes were observed at 24, 48, and 72 hours, and myosin heavy chain was detected by immunofluorescence staining at 48 hours. The fusion index was quantified using ImageJ, and the expression levels of Notch signaling-related genes (Notch1–3, JAG1, JAG2, DLL1, cMYC, HES1, HEY1) were analyzed by RT-qPCR. High concentrations of curcumin (50 and 100 μM) resulted in visible debris accumulation and significantly decreased the fusion index ($p < 0.05$). In contrast, 10 μM curcumin (Cur10) significantly enhanced the fusion index compared to control and DAPT-treated groups ($p < 0.05$). Cur10 also significantly suppressed Notch1, Notch2, Nothc3, DLL1, and cMYC expression ($p < 0.05$), without affecting JAG1, JAG2, HES1, or HEY1. These results indicate that curcumin at optimal concentrations (Cur10) promotes myogenic differentiation by inhibiting upstream Notch signaling and may serve as a natural modulator to enhance satellite cell performance in cultured meat applications.

Abstract # 36

Hydrolyzed Whey Protein as a Functional Alternative to Fetal Bovine Serum for Enhancing Proliferation and Differentiation of Hanwoo Satellite Cells

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Whey, a by-product of cheese and casein production, is often discarded or underutilized despite its high nutritional value, leading to environmental pollution and economic inefficiency. However, due to its rich content of proteins, peptides, and other bioactive compounds, whey can be effectively upcycled into functional ingredients for cell culture systems. This study investigated the effects of partially replacing fetal bovine serum (FBS) with hydrolyzed whey protein (WPH) on the proliferation and differentiation of Hanwoo satellite cells. Cell proliferation assays using MTS and PAX7 staining revealed that treatment groups supplemented with WPH exhibited significantly higher proliferation rates than the control group (F0). Notably, 0.9 mg/mL WPH (F10W9) showed comparable proliferation to the 20% FBS group (F20), suggesting its potential to replace standard FBS concentrations. mRNA expression levels of PAX7 and MYOD1 further supported the proliferative capacity of WPH-supplemented media, with F10W9 showing similar expression to F20. Differentiation capacity was assessed by immunofluorescence staining, myotube area, and fusion index. The F10W6 group exhibited the highest differentiation efficiency, surpassing even F20, whereas F10W9 showed reduced differentiation, likely due to nutrient excess disrupting metabolic regulation. Expression levels of MYOG and MyHC mRNA corroborated these findings, with F10W6 demonstrating the highest MyHC expression. These results indicate that hydrolyzed whey protein, particularly at 0.9 mg/mL, can effectively support the proliferation of Hanwoo satellite cells, and at 0.06 mg/mL, can enhance their differentiation. Thus, WPH has the potential as a functional supplement to reduce FBS dependence in muscle cell culture systems.

Abstract # 38

A method for evaluating the degree of Adipogenic differentiation of porcine cells cultured in suspension based on deep learning

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Fat plays a critical role in determining the quality of meat and meat products. In the context of cultured meat production, adipocyte differentiation via cell culture is a vital process. Traditionally, the evaluation of adipogenic differentiation has relied on quantitative methods such as fluorescence staining image analysis or molecular biology techniques. However, these approaches are often time-consuming, labor-intensive, and subject to observer bias. To address these limitations, we developed a faster, more intuitive, and accurate assessment method by combining high-throughput imaging with deep learning techniques.

Using bright-field images captured throughout the differentiation of porcine suspended adipocytes, we generated two datasets comprising high-throughput bright-field images and Bodipy fluorescence intensity measurements based on our established cell differentiation protocols. For cell detection, we applied the YOLOv7 model, while the ResNet model was employed to predict fluorescence intensities. Validated through datasets from both single-flask and multi-flask cultures, our combined approach demonstrated robust performance, achieving a root mean square error (RMSE) of 5.90 and an R^2 of 0.8321.

We established a standardized metric for adipogenic differentiation by converting mean fluorescence intensity values through min-max normalization. Our model effectively screened 18 adipogenic differentiation factors and shows promise for future high-throughput screening of additional factors. Leveraging this integrated platform of high-throughput screening and deep learning, we identified arachidonic acid (100 μM) as a potential adipogenic differentiation enhancer during rapid screening of edible fatty acids and proteins.

This novel methodology streamlines the evaluation of adipocyte differentiation and facilitates accelerated discovery of functional compounds relevant to cultured fat production.

Abstract # 39

Evaluation of Plant-Based Textured Scaffolds for Myoblast Growth in Cultured Meat Development

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Cultured meat production requires scaffolds that support cell growth and mimic the properties of animal tissue. Low-moisture extrusion is a sustainable technique to create fibrous structures from plant proteins, making them suitable as scaffolds for muscle tissue engineering.
As part of the MEAT4FUTURE project, several textured plant-based scaffolds were developed and assessed using murine and primary bovine myoblasts. Six candidate scaffolds were selected based on visual appearance, fibrous structure, and size uniformity. These samples were sterilized, hydrated, and sliced before in vitro testing.
Two promising scaffolds—F2M2 (based on mung bean protein) and F6.2M3 (a blend of pea protein and yeast)—were selected for further evaluation. Murine myoblasts were embedded in alginate crosslinked with $CaCl_2$ or fibrinogen crosslinked with thrombin. Cell viability was assessed on days 4, 7, and 14. Both scaffolds supported good cell viability, but after thermal processing, F6.2M3 was chosen for its superior cooking performance.
F6.2M3 was then tested with primary bovine myoblasts. Viability and scaffold integrity were monitored over 21 days. F6.2M3 supported long-term cell survival, particularly when combined with the fibrinogen–thrombin matrix. Cooking tests at each time point evaluated texture, shrinkage, and aroma, both with and without cells. Over time, F6.2M3 developed meat-like aromas and improved texture, closely resembling cooked muscle tissue.
These results highlight F6.2M3 as a promising scaffold for cultured meat applications, combining structural fidelity, cellular compatibility, and favorable sensory evolution aligned with cell proliferation.

Abstract # 40

Evaluation of Decellularized Fibroblast-Derived ECM as a Scaffold for Porcine Muscle Stem Cell Culture

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This study investigates the potential of decellularized fibroblast-derived extracellular matrix (ECM) as a scaffold for porcine muscle stem cells (pMuSCs), aiming to provide a animal-free substrate for cultured meat production. Fibroblasts were cultured and decellularized to obtain ECM coatings, which were then compared to non-coated surfaces and surfaces coated with porcine gelatin. The proliferation of pMuSCs was significantly enhanced on the decellularized ECM compared to both controls. During the differentiation stage, immunostaining revealed a higher number of myosin heavy chain (MHC)-positive cells and a significantly elevated fusion index, defined as the percentage of myotubes containing three or more nuclei, on ECM-coated surfaces. These findings suggest that ECM derived from fibroblasts provides pro-myogenic cues that better support pMuSC growth and myotube formation than commonly used substrates. However, quantitative PCR analysis of myogenic markers did not show statistically significant differences between groups, indicating that transcriptional changes may not fully reflect functional outcomes, or that further molecular analyses are necessary. Overall, this work demonstrates that decellularized fibroblast ECM not only supports the proliferation and differentiation of porcine muscle stem cells but also holds promise as a natural, animal cell-derived scaffold material for use in cultured meat systems. Further investigation into the specific ECM components responsible for these effects is warranted.

Abstract # 41

"Smart Cellulose Scaffolds: Revolutionizing Sustainable Cultivated Meat with Efficient Growth Factor Delivery"

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Cultivated meat (CM) represents a promising sustainable alternative to conventional meat production by cultivating animal cells to generate structured meat products. Achieving whole-cut CM with native-like texture requires advanced scaffold materials that support cell growth, differentiation, and nutrient exchange. Here, we present a cellulose-based scaffold fabricated via directional freezing of microcrystalline cellulose, optimized for bovine mesenchymal stem cell (bMSC) culture. This scaffold mimics the extracellular matrix's fibrous structure, promoting parallel alignment and efficient differentiation into muscle and fat lineages, key for marbled meat products. We further introduce a "Smart Scaffold" approach that allows growth factors (GFs) to be attached in a single step, significantly lowering the amount of expensive GFs required for cell growth and differentiation by at least tenfold. Extended culture periods showed strong cell viability and tissue development for up to two months, with mechanical characteristics similar to those of conventional meat. This scalable platform offers a significant reduction in growth factor costs and media consumption, addressing key bottlenecks in CM production. Our findings provide a practical route toward economically viable, sustainable, and ethically sound whole-cut cultivated meat, supporting the advancement of the cultured meat industry and global food security.

Abstract # 43

3D Printing of Hybrid Cultured Meat Constructs Using Plant Proteins and Gellan-Based Fat Inks

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The development of hybrid cultured meat constructs combining edible biomaterials with fat and muscle precursor cells represents a promising strategy for early product prototypes. In this study, we evaluated soy and pea protein isolates as structurally stable bioinks and combined them with gellan gum-based inks containing bovine adipose-derived stem cell (bASC) spheroids. Rheological measurements of pure gellan and protein isolate gels were performed to determine their suitability for extrusion-based printing and post-processing. Following 3D printing, we characterized the stability of the printed scaffolds after cold storage (4 °C) and freezing (–20 °C), as well as their behavior during pan-frying. Color-modified constructs stained with beetroot juice were analyzed for macroscopic appearance, color change, and mechanical properties before and after cooking. While gellan-based constructs melted significantly during frying, soy and pea protein scaffolds retained their structure and exhibited a crispy surface texture. This melting behavior of the gellan phase was intentionally exploited in co-printed constructs to mimic the juiciness of intramuscular fat. Cell-laden gellan inks were successfully printed in adapted CAD-models using a protective outer layer of protein isolate ink. The co-printing approach improved structural resolution and enabled the combination of thermosensitive and stable materials in one construct. Mechanical testing before and after cooking was conducted to assess potential differences between cell-free and cell-laden prototypes. This study demonstrates that the composition and architectural integration of bioinks significantly affect both the technical and sensory properties of hybrid cultured meat products.

Abstract # 44

Could Mycelia Grow Your Next Meatball?

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Efficient expansion and differentiation of adherent cells remain key bottlenecks in the bioprocess development for cultivated meat. Edible microcarriers (MCs) combined with stirred tank bioreactors present a scalable, cost-effective solution, whilst also contributing to the nutritional and sensory profile of the final product. In this study, food-grade filamentous fungi, derived from Myconeos' dairy industry strains, were evaluated as edible MCs. These fungi naturally form spherical, porous pellets with fibrous hyphal surfaces that promote oxygen and nutrient transfer. Their size, morphology and colour can be tailored by adjusting culture parameters. Following an initial screening of eleven strains, four were selected for further testing (Myc1-4) based on cytocompatibility and ability to form uniform, easy-to-handle pellets with high surface area.

Adipose-derived bovine mesenchymal stem cell (bMSC) proliferation was assessed over five days in planar cultures using PrestoBlue assays (1.8 million cells/g MCs). All strains supported attachment and growth in α-MEM media supplemented with FBS. Myc2 showed the highest initial attachment (88.2%), whilst Myc3 demonstrated the strongest proliferation, with a specific growth rate of 0.016 h^{-1} and a 2.35-fold increase by Day 5. To improve industrial relevance, we also tested Proliferum B (ProB), a plant-based FBS alternative from Multus. ProB significantly outperformed FBS across all strains - Myc4 showed a 264% increase in attachment and >4× higher cell density by Day 3. Fluorescent imaging revealed improved morphology and reduced aggregation.

Ongoing work includes optimisation of seeding densities, mechanical characterisation of the top-performing strains and evaluation of MC behaviour under mixing conditions in spinner flasks.

Abstract # 48

Designing Wet-Spun Cultivated Meat Muscle Fibers: The Impact of Faba Protein Isolate Hydrolysis and Concentration

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With the world's population expected to reach 10 billion by 2050, food source diversification is needed to mitigate supply chain vulnerabilities due to climate instability, political volatility, and epidemics. Novel protein foods are one promising approach to diversifying supply chains and production methods. To date, gentle processing methods to create anisotropic whole-cut meat analogs have proven difficult to scale. Wet-spun calcium alginate fibers fortified with macro- and micro-nutrients could prove to be a scalable technology to produce species-diverse muscle tissue analogs. In this study, faba protein isolate was evaluated at different concentrations and hydrolysis intervals to assess the impact on dispersion stability, post-crosslink structure-function characteristics, and multi-species cell-to-fiber viability. Results indicated increasing concentrations of intact faba protein isolate increased fiber water holding capacity, structural irregularities, and thermal degradation temperatures. Hydrolyzed proteins resulted in increased morphological homogeneity and are expected to improve cell viability. Unlike the native proteins, high concentrations of hydrolyzed proteins allowed for a retention of stiffness. Hydrolyzed proteins also decreased water holding capacity and thermal degradation temperatures but likely within an acceptable range for a downstream product. Pending experiments may confirm cell viability improvements for hydrolyzed protein loaded fibers given increased adhesion motifs, peptide availability, and stiffness. The study suggests differing faba protein concentrations and hydrolysis levels allows for optimized cell viability and tunable functional properties at the muscle fiber level for novel protein foods.

Abstract # 58

TERT-activating compound increases the stemness of primary porcine adipose-derived mesenchymal stem cells

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Using primary cells for cultivated meat production encounters several challenges due to their progressive loss of stemness, requiring strategies to maintain their regenerative and differentiation potential.
Recently, the small molecule TERT-activating compound (TAC) has been identified as a potent activator of TERT expression [1]. TAC treatment has also been shown to maintain telomere lengths of primary human fibroblasts and their proliferative capacity [1].
Here, TAC treatment was tested on primary porcine adipose-derived mesenchymal stem cells (pAD-MSCs) in long-term adherent cell culture. The proliferation and differentiation capacity of freshly isolated pAD-MSCs with lower initial population doublings (LPD) and pre-cultured pAD-MSCs with higher initial population doublings (HPD) was assessed over the course of 15 passages.
TAC-treated cells showed a longer linear growth of cumulative population doublings, a slower increase in the population doubling time, and reached higher final CPDs compared to untreated cells. Over multiple passages, the adipogenic differentiation potential of LPD cells gradually decreased, independently of TAC treatment, while the adipogenic differentiation potential of HPD cells further decreased without TAC treatment but was maintained over multiple passages with TAC treatment.
In conclusion, TAC is a small molecule that, when supplemented to the cell culture media, enhances the proliferation and differentiation potential of pAD-MSCs. Therefore, TAC represents a valuable tool for transiently maintaining stemness in primary cells, while complying with regulatory frameworks for novel foods that are less friendly towards genetic modifications.

[1] Shim, Hong Seok, et al. ""TERT activation targets DNA methylation and multiple aging hallmarks."" Cell 187.15 (2024): 4030-4042.

Abstract # 75

Overcoming Scalability and Structure Challenges in Cultivated Meat with Bioinspired Fiber Scaffolds

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We have developed a novel fiber spinning platform that directly addresses key challenges in cultivated meat production, including scalability, structure, and cost. The system produces bioinspired, cell-laden fibers that enable high density, structured tissue growth in a controlled and shear free environment. By optimizing spinning parameters and fiber design, the platform can be tailored to support various cell types and meat formats, replicating the texture and architecture of conventional meat. The device allows independent control of extrusion and fiber collection speed enabling systematic investigation of their effects on fiber morphology and embedded cell performance. Cell laden fibers produced at slower extrusion speeds (≤1 mL/min) and moderate collection speeds (10–20 rpm) showed uniform diameters (200–300 μm) and porous, aligned internal structures, favorable for nutrient diffusion and cell infiltration. Live/dead staining and metabolic activity assays demonstrated that these conditions supported >95% cell viability after 7 days in culture for primary chicken cells embedded during spinning. In addition to maintaining viability, cells were observed to proliferate within the scaffold over time, indicating the suitability of the fiber environment for sustained tissue development. These results demonstrate that the developed platform can reliably produce structured, cell-laden fibers with high viability and tunable properties, offering a scalable and adaptable solution for cultivated meat production.

Abstract # 81

Transforming cell culture media design with AI and automation to design scalable, affordable and animal-free formulations

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The commercialisation of cultivated meat products is currently constrained by the limited availability of low-cost, scalable, and food-safe growth media. To address this, we have developed a novel AI and automation enabled platform for the accelerated development of cost-effective, high performance growth media. Here we present the successful development of serum-free formulations costing under £10/L, capable of supporting cell growth on par with the industry standard foetal bovine serum (FBS), within a five-month timeframe. First, we created a robust ingredient library of 100 ingredients prioritising the sourcing of cost-effective, scalable ingredients that offer bio-functional benefits. Next, candidate ingredients were screened using one-factor at a time (OFAT) and combinatorial assays, generating data on bioactivity and toxicity to inform ingredient selection. Then, over 300 different formulations were created and evaluated on cell growth kinetics and morphology. This dataset was integrated into our multi-objective Bayesian optimisation algorithm to guide the selection of subsequent formulations using an iterative process until optimal growth, morphology, and cost targets were achieved. After three iterations, 90 formulations were selected for extended culture and functional testing to assess their capacity to support long-term proliferation and maintain the desired cellular phenotype. This project generated formulations that rival FBS performance at under £10/L within less than six months from project inception to launch. This case study demonstrates that our refined AI-driven media development approach can significantly reduce media development timelines while delivering cost-effective solutions that align with the industry's growing demand for scalable, affordable, and animal component–free media solutions.

Abstract # 86

Tuning matrix viscoelasticity to control proliferation and myogenesis in cultivated meat production

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Cellular agriculture is a biotechnological field focused on producing traditionally animal-derived products by cultivating cells in controlled environments. Among other applications, it holds the potential to replicate the organoleptic and nutritional properties of natural meat. Despite promising advancements, major challenges associated with efficient muscle cell differentiation are yet to be solved. Current cultures are often heterogeneous, with limited differentiation profile which is hard to control. Often this results in insufficient muscle maturation and low protein expression as a significant proportion of cells do not undergo myofusion.

This project aims to design tailored biomaterials for bovine muscle development in cultivated meat production. We hypothesize that mechanical cues regulate muscle cell behavior and aim to identify optimal conditions that promote cell proliferation and enhance differentiation.

Alginate — a biocompatible, edible, and scalable polysaccharide — is used as the base material. Oxidized alginate (OA), with higher chemical reactivity and tunability, allows modulation of stiffness and stress relaxation properties. Viscoelastic properties were assessed using an HR 10 rheometer (TA Instruments). An immortalized bovine satellite cell line (iBSC1) was cultured on the engineered 3D matrices. Cell proliferation is assessed via confluency, metabolic activity and growth rate. Differentiation is evaluated through myotube formation (immunostaining) and western blot-based protein quantification, targeting sequential markers such as myosin heavy chain, and desmin.

By integrating defined biomaterial designs with a favorable cellular response, this project contributes to developing edible, scalable scaffolds that support higher structured tissue formation — advancing the field of cultivated meat towards high-quality, sustainable protein alternatives.

Abstract # 93

Cell engineering toolkits for cultivated meat

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Development of cultivated meat is held back by intrinsic limitations of animal cells with respect to industrial-scale bioprocesses, including reliance on complex and expensive media, inefficient metabolism, and high sensitivity to shear stress, amongst many others. Tools for efficient modification of endogenous genes and accurate insertion of new ones are essential for the engineering of cell lines with new and improved phenotypes that can surmount these barriers.

To address these challenges, we have developed a toolkit consisting of a broad suite of CRISPR/Cas-based systems, including nucleases, double-nickases, and base editors, for the precise and efficient editing of the endogenous genome in bovine satellite cell lines. We demonstrate the power of this toolkit by targeting the CDKN2A (p16) locus, a key cell cycle regulator whose knockout confers a robust anti-senescence phenotype. In parallel, we are constructing a modular platform for stable genomic integration using Bxb1-mediated recombination. This system enables targeted, high-fidelity insertion of long DNA sequences into pre-engineered ‘landing pad’ cell lines in a simultaneous or sequential fashion, providing a critical tool for the efficient insertion of large, multigenic constructs for conferring various complex phenotypes.

Together, these tools provide a powerful foundation for the rational engineering of bovine satellite cells, opening the door to cell lines that underpin cost-effective, scalable, and sustainable cultivated meat production processes.

Abstract # 94

An Antibody-Free Strategy for Enriching Myogenic Progenitors from Muscle-Derived Cell Populations

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Antibodies are widely used to isolate specific cell types from heterogeneous populations, but their limited availability in non-mammalian species restricts their application in cultivated meat research and production. In particular, the lack of species-specific antibody panels hinders the use of flow cytometry to enrich myogenic progenitors, key cells for muscle tissue formation. While pre-plating procedure is commonly used as an alternative, it offers only modest improvements in purity, especially over extended culture periods.

Here, we present an antibody-free enrichment method based on intrinsic cell size differences. Myogenic progenitors are typically smaller than other adherent muscle-derived cells shortly after isolation. By sorting cells using forward scatter area (FSC-A) in flow cytometry and gating for the smallest population, we achieved significant enrichment of muscle progenitors. Using chicken muscle-derived cells, Pax7 immunostaining confirmed that the proportion of progenitor cells increased from 53.4 ± 22.9% in unsorted populations to 81.2 ± 7.2% (n=4) post-sorting.

This straightforward, species-agnostic strategy provides an efficient alternative for enriching satellite cells in systems lacking flow cytometry-compatible antibodies. Beyond improving progenitor purity, FSC-based sorting delays fibroblast overgrowth and enhances the accuracy of downstream applications in satellite cell biology and myogenesis.

Abstract # 96

TOO HOT TO BURN: INDUCING ADIPOGENESIS WITH HEAT

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Cultivated meat holds great promise as a sustainable alternative to conventional animal products, but several cultural and technical bottlenecks still remain. Among the latter, the development of robust cell lines and the ability to control their differentiation into target tissues, such as muscle and fat, are key challenges. Particularly, fat is a crucial component of meat, contributing to its flavour, texture, and nutritional value. However, in vitro adipogenesis is often slow and typically requires chemical inducers not intended for human consumption. In this context, tools capable of modulating cell fate in a predictable and food-compatible manner are urgently needed. We present a synthetic system inspired by the heat shock response, a natural mechanism that allows cells to adapt to thermal stress. Our patented approach combines a construct that constitutively expresses HSF1 with constructs encoding myogenic or adipogenic transcription factors, placed under the control of a temperature-sensitive Hsp70 promoter carrying a defined number of heat shock elements (HSEs). This design enables precise temporal regulation of gene expression through mild thermal inputs, without the use of potentially harmful molecules. Preliminary data obtained in our laboratory using myogenic master regulators show that HSF1 enhances Hsp70 promoter activity, triggering early myogenic differentiation and accelerating the expression of late myogenic markers, as well as the formation of multinucleated myotubes in myogenic-competent cells. Current efforts focus on applying this strategy to promote adipogenesis in cell lines derived from species relevant to cultivated meat, via temperature-controlled expression of PPARγ and C/EBPβ, two key regulators of adipogenic differentiation.

Abstract # 98

Establishment and optimization of meagre (Argyrosomus regius) cell lines for cultivated seafood applications

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Cultivated seafood (CS) represents a promising alternative to wild fisheries and conventional aquaculture, provided that stable and scalable fish cell lines are developed and culture conditions are optimized for efficiency and cost-effectiveness. In this study, we report the successful isolation and long-term cultivation of multiple cell lines derived from the meagre (Argyrosomus regius), a high-value marine species with limited aquaculture production. Primary cells were obtained from fin, jaw, vertebra, and branchial arches via enzymatic explant digestion and cultured at 22°C in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum (FBS). Several cultures underwent spontaneous immortalization after 40-60 doublings and were maintained for over 120 passages. To enhance scalability and reduce reliance on animal-derived components, we investigated the effects of temperature and FBS concentration on growth performance. Cultures maintained at 33°C showed reduced doubling times, ranging from 12–25 hours, and selected lines continued to proliferate with FBS levels as low as 4%, without compromising viability. Cell lines were further characterized through growth rate, tissue-specific marker expression, and functional assays. A subset of cell lines exhibited adipogenic differentiation, confirmed by lipid accumulation and adipocyte-specific gene expression, while soft agar assays confirmed cells non-tumorigenic nature. These findings highlight the plasticity of fish cells, including spontaneous immortalization. The cell lines established here provide a valuable platform for advancing CS technologies and support sustainable marine protein development.

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Abstract # 99

Engineered inducible differentiation control for cultured meat

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Industrial bioprocesses for cultured meat place high phenotypic demands on their input cells, which must rapidly proliferate to accumulate biomass while maintaining robust differentiation potential for subsequent cell fate commitment. Selecting these traits in native populations can be challenging, as proliferative capacity and lineage commitment often transcriptionally trade off.

To efficiently and robustly mediate the proliferation-differentiation transition, we developed engineered DF-1 chicken fibroblast cell lines that respond to user-defined inputs with transcriptional output driving either adipogenic or myogenic differentiation. When uninduced, the cell lines maintain fibroblast phenotypic hallmarks of their parental cell line, such as indefinite proliferation with a stable doubling time of ~23.8 hours, fibroblast-like morphology, and marker expression. Myogenic transdifferentiation from chicken fibroblasts is driven by ectopic expression of MyoD, with muscle-specific hallmarks such as multinucleation and muscle marker expression detectable within 3 days. Adipogenic transdifferentiation from chicken fibroblasts is driven by ectopic co-expression of C/EBPa and PPARg, identified in this study to more rapidly and robustly activate adipogenesis than expression of either transcription factor alone. In a minimal media containing only oleic acid and rosiglitazone, adipogenic hallmarks such as lipid droplet formation and adipogenic marker expression were detectable within 3 days; strikingly, unilocular adipocytes containing a large, single lipid droplet were widespread as early as 7 days post-induction.

In this study, drug-induction (Tet-On) and contact-induction (SynNotch) systems are used to activate expression of master transcriptional regulators of adipogenic or myogenic cell fate. Drug-inducible behavioral switching enables temporal control over cell fate, and is well-suited to differentiation-in-place bioprocess strategies utilizing a single reactor for both proliferation and differentiation. Contact-inducible switching, on the other hand, enables spatial control over cell fate, facilitating complex tissue patterning. We hope to show that diverse engineered inducers introduce novel layers of cell fate control that can be tailored to specific bioprocess needs.

Abstract # 102

Evaluating the Quality Attributes of Cell-Based Meat Compared to Hanwoo Beef

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This study compared the physicochemical properties of cell-based food (CBF) and Hanwoo beef. Scanning electron microscopy revealed that the surface of CBF became smoother after heating at 60 °C. CBF had a higher moisture content, whereas Hanwoo beef contained significantly greater levels of crude protein, crude fat, and ash. In terms of color, cooked Hanwoo beef exhibited significantly higher L*, a*, and b* values compared to cooked CBF ($p < 0.05$). Texture profile analysis showed that Hanwoo beef had significantly higher values for hardness, gumminess, chewiness, and cohesiveness, whereas CBF had lower values for these parameters. Springiness was comparable between the two. Additionally, Hanwoo beef contained higher concentrations of amino acids than CBF. Further research is needed to explore additional characteristics of CBF, including edible scaffolds, metabolite profiles, flavor attributes, and safety-related components.

Abstract # 104

Biofabrication of Structured Cultivated Meat: High-viscosity Bioinks Towards Fibrous Texture and Myogenic Differentiation

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Cultivated meat is emerging as a sustainable alternative to conventional animal agriculture; yet replicating the structure and texture of whole-cut meat at scale remains a major challenge. Achieving a broader real-world impact requires scalable strategies that recreate the hierarchical organization and mechanical properties of native (e.g. bovine) muscle tissue. Our research aims to address this by applying extrusion-based 3D bioprinting to engineer structured muscle tissues with fibrous alignment and meat-like texture. Our approach focuses on developing animal-free, fibrillar bioinks with tuneable mechanical and biological properties. We bioprint hydrogel scaffolds with shear-induced fibrillar alignment to provide anisotropic cues for aligned myogenic differentiation in 3D. A pilot formulation of oxidized-alginate with food-grade viscosity enhancers (methyl cellulose or ι-carrageenan) is evaluated for rheology, print fidelity, and cytocompatibility. Inks containing 3% oxidized alginate and 3-9% thickeners exhibited shear-thinning behaviour; notably, 6% methyl cellulose yielded good print fidelity (filament diameter <300 µm) on the GESIM BioScaffolder 3.1. Scaffold geometry is analysed via stereomicroscopy, and biological performance is assessed using C2C12 myoblasts with immunofluorescence. To replicate fibrous textures, micro-/nanofibers are incorporated into the inks. Single-layer filaments are printed under varying parameters, and fibre alignment is validated using (polarized) light and fluorescent microscopy. C2C12 myoblasts are induced to differentiate on these aligned 3D printed filaments, with myotube formation and orientation evaluated via immunostaining. Towards scalable production of cultivated meat alternatives in the future, tuneable bioink formulations and bioactive components are investigated to enhance cell-material interaction for improved myogenic maturation in larger 3D bioprinted constructs.

Abstract # 105

Wnt-driven hypertrophy: fine-tuning myogenesis for increased protein yield

Lucy Wilkinson

University of Bath

For cultivated meat to replicate the nutritional and sensory qualities of conventional meat, protein production in muscle-derived cell lines must be optimised. Multiple studies have described the induction of in vitro muscle hypertrophy, characterised by an increase in muscle mass due to increased myofiber fusion and size, through canonical and non-canonical Wnt signalling, particularly downstream of Wnt7a and its receptor. This project aims to induce and characterise a hypertrophic response in in vitro myotubes in agriculturally relevant species using Wnt pathway modulators and anabolic signalling activators. To date, our work shows that Wnt7a treatment increases expression of myogenic markers MyoD and myogenin, and increases overall protein content following a 6 day differentiation period. Data from this work will inform future cell line development strategies, namely overexpression of Wnt signalling proteins, to improve the temporal control and efficiency of in vitro myogenesis for cultivated meat production.

Poster Session B

Abstract # 2

Designing a platform process for cultivated meat products

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Cultivated meat (CM) is viewed as a sustainable and animal-friendlier alternative to traditional meat production. Although different companies and research groups are working to scale up the production of CM, commercial scale processes are still hindered by high production costs resulting from low cell titers, expensive growth media, and scale up complexity. A platform process could enable the manufacture of several CM products in a defined production system, increasing process agility, efficiency, and profitability. Platform processes are well established for e.g. biopharma, but this concept has not yet been assessed for CM.
To foster the transition towards cellular agriculture, this work has explored the design of a platform process for CM products, with a comprehensive identification of best-performing process scenarios, and a focus on techno-economic assessment.
Aiming at the annual production of 10 kiloton CM, process configurations have been assessed considering reactor type, operating mode, downstream processing steps, and formulation techniques for both structured and unstructured products. Airlift bioreactors have been favored due to their scalability and cost. Scenarios considering non-GMO and GMO metabolic targets have been investigated for cultured muscle cells, animal fat cells, and their combined production. Bioreactor performance has been modelled accounting for oxygen and carbon dioxide limitations, along with ammonia and lactate inhibition. Overall techno-economic feasibility and environmental impact have been estimated using SuperPro Designer.
Production costs between 8–30 €/kg have been estimated, with process profitability highly dependent on cell metabolic performance and product selling price. The non-GMO scenarios could not be proven profitable for selling prices between 12–30 €/kg, mostly due to high media requirements and media-related costs. Conversely, a preliminary environmental assessment has suggested advantages compared to traditional animal husbandry, with lower global warming potential, water and land usage for all the scenarios considered.

This project has been funded by the Nationaal Groeifonds via the Cellular Agriculture Netherlands Foundation (CANS).

Abstract # 3

Cultivation of animal proteins as three-dimensional structures: modelling and simulation studies

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Cultivated meat (CM) offers a sustainable alternative to conventional meat, while preserving the nutritional and organoleptic properties desired by consumers. To manufacture structured, whole-cut CM products, it is convenient to grow cells on scaffolds, successfully mimicking the cytoarchitecture of meat. However, such three-dimensional (3D) structures require an efficient and constant perfusion of oxygen- and nutrient-rich media, and the cells typically experience shear stress and mass transfer limitations. These challenges limit process scalability, as well as applicable choices for scaffold materials and scaffold and bioreactor types, leading to reduced scaffolded tissue and cell titers to maintain cell viability. To accelerate the affordable production of 3D CM products, cost-effective scalable process configurations need to maximize cell growth while minimizing shear stress and mass transfer limitations.
This work investigated the physical limits for scaling up the production of 3D CM using scaffolding materials, aiming at the annual production of 1 kiloton structured product. Edible hollow fiber reactors have been chosen due to their technical feasibility. A mathematical model has been developed to optimize independent process parameters, and identify physical limits and trade-offs between reactor scale, yield, product quality, and costs. Preliminary results suggest that process scalability and costs are highly sensitive to fiber permeability and rupture tolerance. The maximum achievable cell titer is limited by the fiber wall thickness and oxygen penetration depth. Product quality depends on the fiber lumen diameter, since larger diameters lead to lower CM content, but may allow for larger bioreactors, denoting the evident trade-offs between product quality and process scalability. A comprehensive sensitivity analysis has provided further insights into favorable fiber material properties, cell kinetics and stoichiometry targets, and opportunities for process optimization, to serve as valuable guidance for future research within the cellular agriculture community.

Abstract # 6

Green and scalable method for cultivating fats for food

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Climate change and food security concern are major pain points for livestock agriculture. Rearing cattle and pigs for meat contribute greatly to greenhouse gases emission which can have spiraling negative effect on the agriculture itself. Furthermore, countries that are heavily reliant on trade for its food are exceptionally vulnerable on the availability and supply chain for livestock agriculture. For example, Singapore is small a country that need to import 90% of its food. Functioning supply chain is imperative to the nation. In order to build a more resilient future, many nations have turned to self-sustaining technologies such as lab grown meat. Lab grown meat offers exciting opportunities to tackle these challenges. Lab grown meat harvests only cells from animal of interest and culture these cells into greater numbers and induce them into edible meat tissues such as muscle and fats in a very space efficient manner. This process circumvents the issues presented by current livestock agriculture. Conventional cultivation method involves growing harvested animal cells on plastic flasks, which have limited scalability, low yield and contributes to plastic waste. Our lab has developed a green and scalable method that involves using natural sustainable plant-based 3D scaffold to cultivate cells into fats in an entirely edible system. Our system boasts superior yield compared to conventional method and shares similar composition to pork fat; thereby leading away the reliance from unsustainable livestock agriculture practice.

Abstract # 8

Cultivating Consumer Research: A Systematic Review and Research Agenda on Cultivated Meat Acceptance

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Cultivated meat (CM) is increasingly referenced as a potential solution to several pressing challenges facing current food systems. As CM products potentially advance toward commercialization, understanding the factors driving consumer acceptance becomes critical to ensuring their market success.
Despite a growing body of primary and secondary research, evidence on CM acceptance remains fragmented due to methodological inconsistencies and theoretical divergences. These limitations hinder cross-study comparability, reduce the generalizability and replicability of findings, and ultimately constrain the development of robust conclusions in this field.

To address these issues, this systematic literature review provides a comprehensive synthesis of the existing research on consumer acceptance of CM. Conducted following PRISMA guidelines, the review examines theories, contexts, characteristics, and methodological approaches of 250 peer-reviewed articles on CM acceptance, making it the most extensive synthesis on this research topic to date.

Preliminary findings point to major limitations in the current evidence base, such as the predominant reliance on WEIRD (White, Educated, Industrialized, Rich, and Democratic) samples, with limited representation from regions expected to experience the highest demographic growth in the coming decades. Further barriers to comparability and replicability include inconsistent nomenclature of CM, data recency limitations, and a widespread dependence on non-representative samples.

By identifying these critical gaps, the review outlines a roadmap for future consumer research on CM acceptance, emphasizing the need for broader geographical coverage and stronger methodological rigor. In doing so, it draws attention to several underexplored yet essential areas that demand urgent scholarly attention and empirical inquiry.

Abstract # 9

Meat or non-meat? Public perceptions of cultured 'meat' in Japan and the United Kingdom

Aiko Hibino

Hirosaki University

The aim of this study is to clarify how perceptions of cultured meat are influenced by cognitive views of life through the questionnaire surveys conducted in Japan and the United Kingdom. The development of cellular agriculture technology has made it possible to produce many products, including food, using cultured cells from quails and eels. Some argue that cellular agriculture products do not necessarily need to be labelled as 'meat'. While surveys on consumers' acceptance of cultured meat have been conducted in many countries, few have focused on the use of the term 'meat'. Such views are affected by cultural factors, such as cognitive views of life and dietary habits. We conducted questionnaire surveys of 1200 respondents between the ages of 20 and 59 in Japan and the UK. The percentage of those in favour of using the term 'meat' for cell-based food was relatively low in Japan (41.5%), but high in the UK (57.5%). Multiple correspondence analyses were conducted to classify respondents' opinions into clusters. The results showed that those who agreed with the use of the term 'meat', in both countries, were more likely to try cultured meat, to be more flexitarian in their dietary habits, to be more environmentally conscious and to have a relational view of life. Specific dietary preferences of vegetarian were less associated with this differentiation of opinions. Our results suggest the effectiveness of framing cell-based products as meat to enhance their acceptance.

Abstract # 17

Optimisation and scale-up of serum-free or low-serum media formulations for proliferation of murine and porcine myosatellite cells using scalable and food-grade inputs

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The development of scalable, low-cost, and food-grade proliferation media is critical for advancing the feasibility of cultivated meat. This study employs high-throughput screening and Design of Experiments (DoE) methodologies to optimise serum-free formulations using affordable, scalable ingredients. We evaluated 42 candidate components, including recombinant proteins, antioxidants, amino acids, small molecules, protein extracts, and macromolecular crowders.

For C2C12 cells, the food-grade Mus musculus Medium 1 (MMM1)—containing spirulina extract and 0.1 mg/mL methylcellulose—achieved comparable growth to serum-containing controls (20% FBS + 5 ng/mL FGF2) in 2D culture over multiple passages, with an average population doubling time of 15.57 hours. MMM1 also outperformed traditional growth media (GM) in microcarrier cultures.

For primary porcine satellite cells, fully serum-free formulations did not sustain long-term proliferation. However, serum-reduced conditions (2% FBS) enabled multi-passage expansion and microcarrier growth at rates similar to GM. Key formulations (SSM3 & SSM4) incorporated food-grade macromolecular crowders and small molecules (forskolin, ostarine).

This work establishes a media optimisation pipeline for multiple cell types, identifies promising novel ingredients, and validates formulations for further serum-free research. However, improved serum-free cell attachment methods remain essential for fully serum-free culture of primary porcine myosatellite cells.

Abstract # 18

Evaluating Food-Grade Growth Factors in the Culture of Stem Cells for Cultivated Meat

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Background
Pluripotent stem cells (PSCs) from food-relevant species are attractive for cultivated meat production because they self-renew indefinitely and can differentiate into both adipocytes and myocytes. Roslin Technologies has established porcine and ovine induced PSC (iPSC) lines, bovine embryonic stem cell (ESC) lines, and salmon progenitor lines, all of which depend on fibroblast growth factor-2 (FGF-2) for proliferation. Replacing pharmaceutical-grade FGF-2 with a food-grade alternative is essential for cost-effective scale-up and regulatory compliance.

Objective
To evaluate novel, food-grade, species-matched FGF-2 proteins developed by dsm-firmenich as functional replacements for pharmaceutical-grade FGF-2 in the routine expansion of livestock PSCs.

Methods
Recombinant bovine and salmon FGF-2 variants were produced in food-approved microbial hosts and recovered through a simplified purification workflow. Their performance was tested across Roslin Technologies' cell lines. The first evaluation was performed with porcine iPSCs over five serial passages. End-points included cumulative population doublings, viability, morphology and expression of core pluripotency markers (OCT4, SOX2, NANOG).

Results
- Both bovine and salmon FGF-2 variants sustained robust proliferation and preserved pluripotency in porcine iPSCs for ≥ 5 passages.
- Porcine iPSCs cultured with dsm-firmenich FGF-2 preserved morphology and pluripotency profile by qPCR and flowcytometry analysis.
- Growth kinetics showed minimal batch-to-batch variability across the eight FGF-2 preparations tested (<8% coefficient of variation in cumulative cell yield).

Conclusion
Food-grade, species-specific FGF-2 from dsm-firmenich matches the performance of pharmaceutical-grade reagent while enabling cost, regulatory and operational advantages – particularly weekend-free feeding – thereby advancing the commercial scalability of PSC-based cultivated-meat processes.

Abstract # 22

Comparative Flavor Profiling of Cultured Meat and Plant-Based Proteins

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Cultured meat (CM) is increasingly regarded as the most promising alternative protein source for replicating the sensory attributes of conventional meat. In this study, we systematically evaluated the physicochemical and flavor characteristics of meat analogs formulated with different primary protein sources: cultured meat (CM; derived from bovine muscle stem cells), myofibrillar protein (MP), isolated soy protein (ISP), and textured vegetable protein (TVP). Each analog was prepared with 80% of the target protein source, with moisture content adjusted to a standardized level. Beef tenderloin (TL) served as the reference sample. Basic processing and colorimetric properties were assessed to evaluate cooking performance and visual appearance. Although results varied among protein types, CM exhibited relatively low cooking loss and moderate textural properties. In addition, both raw and cooked CIE color values for CM were generally more comparable to those of TL than the plant-based counterparts. Compared to beef, flavor wheel analysis based on volatile compound profiling indicated that CM showed the most similar flavor profile. In CM and beef, the proportions of fruity, grassy, and malty note were similar, while CM showed relatively stronger sweet and fatty note. In the case of ISP and TVP, which are plant-based proteins, the flavor wheel showed a different flavor pattern from that of beef. Especially, it was showed that grassy and sweet note were very strong and the ratio of fruity note was reduced compared to beef. These findings demonstrate that cultured meat more effectively reproduces the organoleptic properties of conventional meat than current plant-based formulations, reinforcing its potential as a next-generation alternative protein source.

Abstract # 31

Analysis of the economic viability and environmental impacts of a conceptual process for the recovery of lactic acid from spent media in cultivated meat production

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The scaled production of cultivated meat will generate large volumes of liquid waste, primarily in the form of spent media. Recycling this waste—specifically abundant metabolites like lactic acid—offers a potential pathway to reduce environmental impact and improve economic viability. In this study, we present the conceptual design for a five-step lactic acid recovery process, integrated into a previously modeled cultivated meat facility. The process aims to produce 88% aqueous, polymer-grade lactic acid from spent media containing lactic acid at a concentration of 3 g/L.

We evaluated both the economic and environmental impacts of this recovery system and benchmarked the results against commercial lactic acid fermentation data. Our analysis estimates a recovery cost of $0.71 per kg of lactic acid, with a simple payback period of 7.5 years. Sales of recovered lactic acid could offset cultivated meat production costs by $0.06 per kg. Life cycle assessment results showed that the recovery process could either slightly reduce or modestly increase the environmental burden depending on allocation approach, with net effects ranging from –1.0 to +0.2 kg CO_2-equivalent and –22 to +3 MJ in cumulative energy demand per kg of cultivated meat.

These findings suggest that lactic acid recovery from spent media could be both economically viable and environmentally advantageous under the right conditions. This work highlights the potential of metabolite valorization strategies in cultivated meat production and lays the groundwork for broader media recycling practices across animal cell biomanufacturing platforms.

Abstract # 33

Enhanced Purity and Myogenic Differentiation of Hanwoo Muscle Satellite Cells through Cold Shaking Separation

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In this study, we applied an adhesion-based sorting strategy for Hanwoo muscle satellite cells (HMSCs), utilizing their relatively weak adhesion properties compared to other cell types. Three experimental groups were established: (1) Unsorting (US) – cells directly isolated from muscle tissue without further treatment; (2) Pre-plating (PP) – cells cultured at 37°C for 1 h, and only non-adherent cells were collected; and (3) Cold Shaking (CS) – cells from the PP group were further cultured for 24 h, followed by cold PBS treatment and shaking on ice at 150 rpm for 30 min to collect non-adherent cells. Centrifugation-based assessment of adhesion force tests confirmed that HMSCs exhibit lower adherence compared to Hanwoo fibroblasts. MTS assay conducted on day 4 of proliferation showed the highest cell viability in the US group. RT-qPCR analysis revealed that PAX7 expression was highest in PP, followed by CS and US, while MYF5 was most expressed in CS. PDGFRA, a fibroblast marker, showed the highest expression in the US group, indicating lower purity. Upon differentiation, immunofluorescence staining for myosin demonstrated that the CS group exhibited the largest myotube area and highest fusion index. Furthermore, MYH2 protein expression analyzed by Western blotting was highest in CS, followed by PP and US. These findings suggest that the CS method efficiently enriches HMSCs with superior myogenic potential and minimal fibroblast contamination, offering a practical approach for high-purity satellite cell sorting method.

Abstract # 35

Food Chain Structure and Features in the Cultured Meat Industry

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Cultured meat products remain in limited commercial distribution, and the industry has not yet reached a stage where profitability can be realized through product sales. Nevertheless, a growing number of businesses—from established firms in food processing, pharmaceuticals, and agricultural materials to start-ups—have entered the cultured meat sector, actively engaging in research and development. The production of cultured meat involves a series of stages: seed cell extraction, cell cultivation, and molding into final products. This process necessitates key inputs such as culture media and growth factors, as well as infrastructure for large-scale production. Given its reliance on the integration of various technological components, the industry exhibits structural similarities to processing and assembly sectors, such as the automotive industry.

While a segment of consumers remains skeptical due to the perceived artificiality of cultured meat, it is important to recognize that conventional livestock production is also highly industrialized. Intensive animal farming, characterized by large-scale, tightly controlled environments aimed at minimizing costs and maximizing productivity, has long underpinned the economic foundation of the traditional meat industry.

This study conceptualizes the series of production, processing, distribution, and sales processes in the cultured meat industry as a food chain. It seeks to elucidate the structural characteristics of this emerging sector by comparing it with the conventional livestock-based food chain and with supply chain models in other industries. Through this comparative analysis, the paper aims to identify the distinctive attributes of the cultured meat industry, particularly in relation to chain configuration and spillover pathways in production.

Abstract # 37

Valorizing Dairy By-Products: Colostrum Whey as a Sustainable Alternative to Fetal Calf Serum in Muscle Cell Culture

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The development of cultured meat requires scalable, cost-effective, and ethically acceptable cell culture systems. A key limitation remains the reliance on fetal calf serum (FCS), which is costly, variable, and raises ethical concerns. In this study, we evaluated the potential of dairy by-products—specifically whey derived from raw milk and colostrum—as FCS replacements for the culture of C2C12 murine myoblasts.
C2C12 cells were cultured in various basal media supplemented with either raw milk whey or colostrum whey for 4 days to identify serum-free conditions supporting short-term proliferation. The most promising formulation, based on RPMI 1640 and colostrum whey (termed Colostral Medium, CM), was further tested in long-term (30-day) culture to assess sustained proliferation and retention of myogenic potential. Additional experiments evaluated the capacity of CM to support cryopreservation and 3D spheroid culture.
Colostrum whey in RPMI 1640 supported robust proliferation over 4 days, though at lower levels than FCS. CM maintained long-term cell proliferation with a doubling time of roughly 25h (FCS-containing: 20h) and allowed for successful myogenic differentiation post-expansion. CM also enabled effective cryopreservation without FCS. In 3D cultures, spheroids maintained in CM exhibited a marked increase in size and DNA content over 14 days, outperforming spheroids grown in FCS-containing media.
Our findings demonstrate the potential of colostrum whey as a serum-replacement in cell culture of myoblasts. As a low-cost and underutilized by-product, colostrum whey holds promise for advancing sustainable and cost-effective bioprocesses in muscle tissue engineering and cultured meat production.

Abstract # 42

kLa determination in serum-free DMEM/F12 medium using the dynamic pressure method in a 1.5L STR

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Cultivated meat, produced by cultivating mammalian cells in controlled environments, promises a reduced environmental impact compared to traditional meat. To achieve that, efficient production at a large scale is required, and fitting bioreactor design is imperative. Because mammalian cells are known to be sensitive to mechanical and bubble-induced shear stress, stirring speed and aeration rate should be minimized. However, this also reduces the gas-liquid transfer rate, compromising the amount of oxygen available for cellular uptake. To balance supply of oxygen and minimization of shear stress, accurate determination of the mass transfer coefficient (kLa) is essential. It is known the mass transfer coefficient is sensitive not only to operating conditions, but also surface-active compounds in the broth.

In this project the oxygen mass transfer coefficient is determined for a range of agitation and sparging rates in a 1.5L benchtop bioreactor equipped with a Rushton and marine impeller configuration using the dynamic pressure method developed by Linek et al. (1989), and simplified by Scargiali et al. (2010). The kLa is evaluated in PBS as a model for non-coalescent media formulations, and compared with a serum-free growth medium for bovine satellite cells based on DMEM/F12 with and without supplementation of 0.1 g L-1 poloxamer 188. The data generated in this project can contribute to future bioreactor design and optimization studies specifically catered to the needs of cultivated meat.

Abstract # 45

Aroma analysis: a powerful tool to support innovation in food industry

Matthijs Verstraeten

RIC resolve

There is an increasing demand for animal source proteins which leads to an enhanced pressure on the global climate, health, food security, and biodiversity. This triggered research into development of more sustainable ways of producing foods and food ingredients. As a result, alternative manners of delivering proteins to consumers are being developed including plant-based products, cultivated meat, and proteins produced via microbial fermentation (such as biomass and precision fermentation). While the availability of these alternative proteins has greatly expanded over the past years, there is still significant room for innovation especially on acceptance by the consumers. Extensive research is ongoing on understanding how to make the alternative foods scent and taste more like the conventional foods. This is a real challenge and aroma analysis is of paramount importance. Accurate aroma profiling can help manufacturers ensure high quality through proper selection of raw materials and food processing conditions. Therefore, advanced analytical techniques providing improved aroma compounds extraction, separation and identification are required. In this presentation an overview of different untargeted aroma profiling approaches will be presented. These are based on the implementation of green, robust, efficient, cost-effective, and sensitive analysis methodologies. The performance of the applied aroma profiling approaches is illustrated with different conventional and alternative food samples.

Abstract # 50

#cultivatedmeat: Online and social media perceptions of cultivated meat in the United States

Olivia Grieco

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In 2024, cultivated meat was mired in a regulatory battle in the United States, resulting in bans enacted in two states. The bans in Florida and Alabama, along with additional restrictive legislation elsewhere, are largely due to the perception that cultivated meat is “unnatural” and a danger to farmers. However, public perception on cultivated meat remains mixed. While some consumers see its potential for offsetting many of the negative externalities of traditional animal agriculture, most remain unfamiliar while others are skeptical of its healthfulness. Understanding public perception is key for the future of the industry, especially as research and development continue. This study aims to capture individuals’ online and social media perceptions of cultivated meat in the United States to further the understanding of public perceptions and analyze publicly voiced sentiments of the industry. Additionally, it seeks to compare sentiments on cultivated meat both before and after implementation of the bans, and to evaluate differences in sentiment comparing mainstream news media to social media. Using Quid, an online media listening platform, to search and analyze online and social media in 2024 resulted in a total of 374,124 mentions. X/Twitter was the largest domain for mentions. Results indicate that perceptions of cultivated meat across social and news media were largely negative, with large volumes of negative sentiments spiking around the time of each ban. These findings highlight the resultant online discord, negative sentiments, and prolific information dissemination of cultivated meat following bans in the United States.

Abstract # 51

Gen Z Consumer Acceptance of Cell-Cultivated Proteins in Developing Countries: Evidence from Nigeria

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Cell-cultivated meat has been considered by many to be a more sustainable and humane food product compared to conventional meat due to its potential to reduce environmental impacts and improve animal welfare. After receiving its first regulatory approval for sale in Singapore in 2020, it has continued to gain approval in other countries. With rapid and ongoing technological advancements, it is expected to become increasingly accessible worldwide, including in developing countries – yet evidence from lower-middle income countries is absent from the literature. Using Nigeria as a case study, this research examines the purchase intentions of Gen Z consumers toward cell-cultivated meat and seafood, and how various factors influence their purchasing intentions. Data were collected through an online survey of 725 Gen Z consumers in Nigeria in 2023. A majority of respondents indicated excitement to try cultivated meat. Results indicate that consumers who are more open to trying new and unfamiliar foods and those who prioritize cost and animal welfare in their food choices are more likely to try cell-cultivated meat and seafood. Conversely, those with greater objective knowledge of these products are less likely to do so. These findings highlight Gen Z consumers purchase intentions toward cultivated meat and seafood in Nigeria and offer insights for policymakers and retailers who are seeking to promote cell-cultivated meat in these markets.

Abstract # 52

Optimisation of serum-free cell culture medium for porcine preadipocytes

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The cost of cell culture media is one of the main bottlenecks for the mass production of cultivated meat. Developing cheap, serum-free media formulations rapidly for poorly characterised cell lines is essential for the development of accessible cultivated meat. At Hoxton Farms, we combine laboratory automation with design of experiments (DOE) and Bayesian optimisation for the development of cheap, serum-free media. Using space-filling DOE, we showed sequential reductions of media cost in three consecutive iterations, with successive reductions of 59%, 54% and 25% in media cost in each iteration, while simultaneously eliminating serum from our media. We then applied Bayesian optimisation to decrease the price of our serum-free media by a further 52% – ending up with a media 93% cheaper than the original media. In conclusion, using only four rounds of media optimisation and cell line adaptation, we developed a porcine preadipocyte cell line adapted to a serum-free medium, with phenotypic properties similar to the high serum cell line and no loss of adipogenic differentiation capacity.

Abstract # 54

Optimization of Serum-Free Media for the Proliferation and Differentiation of Chicken Muscle Stem Cells

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Serum-free media are crucial for cultured meat, as they enhance food safety, reduce production costs, and enable better control over product quality. Although many serum-free media have been optimized for mammalian muscle stem cells, there is limited research focusing on avian muscle stem cells. This study aimed to formulate serum-free media specifically optimized for proliferation and differentiation of chicken muscle stem cells (cMuSCs) isolated from 18-day-old embryos. We used WST-8 assays to systematically test which of 11 common supplements are necessary for cell proliferation by removing them one at a time from a full serum-free mix. As a result, we identified five key supplements that together formed a simplified serum-free medium capable of supporting strong cell proliferation. We further optimized the concentrations of these five supplements and established a basal serum-free medium based on the optimized levels. For differentiation, cells were first grown in 10% FBS for 3 days, then transferred to media with FBS, HS, or KnockOut™ Serum Replacement (KSR) at 2%, 5%, or 10% concentrations for another 3 days. Among the KSR groups, 2% KSR showed differentiation efficiency, based on mRNA expression of differentiation markers (MYOG, MYH1) and MHC-positive cell ratios, comparable to that of FBS- and HS-based media. These results indicate that 2% KSR can effectively replace serum in promoting myogenic differentiation. Overall, this work presents the groundwork for creating a chemically defined, serum-free system that supports both proliferation and differentiation of cMuSCs.

Abstract # 55

Hinokitiol enables culturing cells without transferrin in serum-free media

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Cultivated meat is currently impossible to provide at moderate costs due to the amount and expense of the serum-free media supply, especially because of the high costs of transferrin, insulin, and growth factors. We have been conducting research to replace these media components to other materials with maintaining their effectiveness. In the first step, we focused on transferrin, which is expensive and has a large market. In cell culture, transferrin is involved in the transport of iron ions to cells. We have discovered that hinokitiol, known for its role as an iron ionophore and its natural products are approved as a food additive in Japan, can function as a transferrin replacement in serum-free culture media. We constructed a cell growth assessment system using an immortalized bovine satellite cell line. Using this system, we found that hinokitiol acts as a transferrin replacement when added to serum-free culture media. This discovery opens a new avenue for providing serum-free culture media and implementing cultivated meat.

Abstract # 56

Nutritional value of cultivated salmon cells – understanding the mechanisms of protein accumulation in fish cells for applications in product development

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What if we could offer special nutritive-rich products for specific target groups in the future? Developing a cultivated fish product with elevated nutritional value requires the establishment of fish cells lines enriched in nutrients such as proteins and lipids. Therefore, understanding the correlation between food and cells culture analytics, as well as investigating the molecular pathways underlying protein and lipid production and accumulation of fish cells are crucial steps for the development of enhanced nutritional cell lines that can ensure resources for food applications.

Methods:
To evaluate the translatability of cell analytics to food analytics, the protein content analysis of small cell culture and tissue samples with BCA assay was correlated with the total amino acid content analysis via LC-MS/MS and NIR-Spectrometer analysis of the same samples (n=36).
To better understand the molecular processes occurring during protein enrichment of fish cells, differential expression and gene enrichment analysis were conducted in different samples of fish cell lines: proliferating muscle derived cells were compared with muscle derived cells that enrich protein without terminal myogenic differentiation.

Results:
Key findings indicate that despite a strong linear relationship between BCA and LC-MS/MS measurements, substantial variability was observed. A calibration equation was used to estimate LC-MS/MS-equivalent protein values from BCA data.
Also, by comparing the gene expression patterns of cells at different stages of protein enrichment, we were able to characterize key genes involved in molecular pathways and cell phenotypes of muscle differentiation and protein accumulation.

Abstract # 59

The Double-edged Sword of the Cultivated Meat Hype: Insights from a Media Analysis in the Netherlands and Germany

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This contribution stems from an EU-funded project, PRISM-LT, and focuses on public perceptions of cultivated meat (CM). It reflects on methodological approaches to identify CM hype and examine its potential impact through a comparative media analysis from the Netherlands and Germany.
The empirical analysis draws from a dataset comprising websites of start-ups, online comments, mass media articles and videos (from Germany and the Netherlands), political statements (with a focus on Germany), and reports based on Technology Assessment (TA), a form of institutionalized scientific policy advise). Qualitative findings suggest pronounced differences between start-up self-presentations, media reports, TA documents and political discourse, but also different streams within these discourse arenas.
For example, mass media reporting is characterized by pragmatism with a tendency towards optimism, but also gives significant attention to polarizing events, and occasionally, features coverage of strong advocates. Online comments on media articles are, overall, more negative, often expressing mistrust in institutions and associations with negatively perceived technologies, dystopian narratives, conspiracy theories, and (targeted) misinformation. However, a small majority of commenters are open, optimistic, or neutral. TA points out uncertainties and provides vague recommendations, giving an overall impression of being very cautious with regard to promises around CM.
The contribution also discusses how these arenas might interact and the implication such interactions hold for the innovation and societal embedding of cultivated meat.
The study concludes by suggesting that future research on the effects of hype would benefit from more experimental approaches and time-sensitive analyses to gain robust insights into the interactions of diverse discourse dynamics.

Abstract # 60

Timescale Analysis for Bioreactor Scale-Up in Cultivated Meat Production

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To enable commercial-scale cultivated meat production, efficient scale-up of the cell expansion phase is essential. However, bioreactor scale-up often leads to productivity losses due to mass transfer limitations, shear stress, and intra-reactor heterogeneity.

This study presents a first-principle analysis of bioreactor designs by comparing timescales of key mechanisms across scales, such as the timescale of oxygen consumption, oxygen transfer and mixing time. Monte Carlo simulations with randomized operational parameters (reactor volume, stirrer speed, gas flow rates, cell density) are filtered using these timescales, forming a framework to evaluate reactor types for large-scale cultivated meat production. The method is applied to stirred tank reactors (STRs) and bubble columns (BCs), with future expansion to airlift and adherent reactor designs.

For STRs, results highlight CO_2 sensitivity and bubble-induced shear resistance as critical parameters. Impeller flooding analysis using critical Flow numbers suggests potential mixing and gas dispersion issues in large-scale STRs with open tube spargers. While microspargers may mitigate this, gas entry velocities required to prevent sparger weeping pose challenges, as indicated by the critical Froude number. Further STR analysis will include variations in geometry (H/D ratio, oxygen enrichment, back pressure) and cell characteristics (growth rate, yield, sensitivities).

For BCs, trends are comparable, but mixing times are significantly longer due to operation in the homogeneous regime at low superficial gas velocities when high sensitivity to bubble induced shear is assumed. This slow mixing leads to large concentration gradients, limiting reactor performance. Circumventing this limitation with shear protectants could open up this operating window.

Abstract # 63

Scaling cultivated food production in hollow fibre membrane bioreactors

Marianne Ellis

Cellular Agriculture Ltd

A major challenge in realising the potential of cultivated food is the manufacturing hardware to produce enough cell mass with the desired nutritional profile cost-effectively. Cellular Agriculture Ltd is developing manufacturing hardware for the scaled production of cultivated foods, initially focused on cultivated meat. The core technology of our bioprocess is a hollow fibre membrane bioreactor (HFB). Here we describe our approach to scaling the HFB, and some key parameters including biomass/cell number yield as well as nutritional data for amino acids, vitamins and minerals. Examples will include data for C2C12 as the ‘workhorse’ cell line and different edible cells, with focus on the change in nutritional data between proliferation and differentiation. The data points to the conclusion that HFBs are suitable for cultivated food manufacturing at the scales necessary for the industry to thrive.

Abstract # 65

A bench-scale hollow fibre bioreactor system for cultivated foods

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As part of our scale-up journey, Cellular Agriculture Ltd has developed a bench scale hollow fibre bioreactor system, that we use internally as part of the scaling process, but has applications as a test-rig for those considering utilising HFB, for scaled-down experiments due to the matched system design or standalone research activities, and for seed-train. Here we present the engineering design and build with key features that make this a great introduction to HFBs for cultivated foods, as well as some output data from our cell database (yield, metabolic data, nutritional data).

Abstract # 68

Public misinformation on Cultured Meat- a thematic analysis of narratives on X (Twitter)

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Cultured meat (CM) has gained attention as a sustainable and ethical alternative to conventional animal agriculture, yet public opinions often reflect resistance shaped by misinformation. This preliminary study investigates how conspiracy-themed misinformation circulates in Twitter (X) discussions on CM and identified narrative patterns that may influence public perception and commercialisation pathways. Over 13,000 tweets were obtained through data mining and analysed using unsupervised machine learning technique for topic modelling-BERTopic. This process resulted in the identification of 267 distinct topic clusters, which served as a starting point for further qualitative interpretation. Topic clusters generated through BERTopic provided a useful foundation, with manual thematic analysis applied to develop meaningful thematic structures through human-led interpretation. Misinformation was identified based on recurring patterns of exaggerated claims, speculative links, and strong ideological language. Three main categories emerged: narratives about elite control, health concerns related to biotechnology, and criticism of economic and political systems. These appeared across multiple tweets and were often expressed in emotionally charged terms. Findings suggest that public narratives around CM include misinformation components that are embedded in broader symbolic and ideological narratives, rather than being purely informational. This study contributes to research on social resistance to food innovation by highlighting the role of misinformation and demonstrating the value of social media analysis in capturing early-stage public narratives around emerging food technologies.

Abstract # 69

Beyond borders: a cross-cultural study on consumer acceptance of cultivated meat between France, Italy, and The Netherlands

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The current animal-based food production system raises concerns due to its misalignment with sustainable development goals from a One Health perspective, impacting human, animal, and environmental health. Cultivated meat (CM) offers a promising alternative to ensure food security by addressing these issues. Significant economic and research investments are being made to develop new CM-based foods. However, understanding and anticipating consumer reactions is crucial for widespread adoption, particularly identifying the psychological factors influencing acceptance.
This study examines consumer attitudes toward CM in Italy, France, and the Netherlands, exploring drivers and barriers to acceptance. A quantitative, cross-sectional design was used, with 500 participants per country, forming representative samples based on gender, age, and education level quotas. Data were collected in 2023 and 2024 through an online self-report questionnaire, using validated scales on food neophobia, technophobia, environmental concern, perceived naturalness, trust in certifying bodies, as well as attitudes, sensory expectations, and intentions to taste and purchase CM.
Analysis revealed significant differences within and between countries, with French consumers showing higher aversion compared to Italian and Dutch counterparts. Although various psychological factors were hypothesized, attitudes toward CM were primarily influenced by food technophobia and trust in food safety authorities, with sociodemographic variations.
This study advances knowledge of CM acceptance, offering new insights through comparisons between European countries. Findings highlight the need for further consumer research on CM acceptance to improve the generalizability of results. Furthermore, insights inform the development of targeted interventions to improve CM consumer perceptions, tailored to cultural and individual specificities.

Abstract # 72

Developing a defined lipid mixture for fish cell suspension culture

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Lipids are critical components of cell membranes, which influence cell proliferation, differentiation, and metabolic activity. In conventional cell culture applications, fetal bovine serum (FBS) supplies the cells with a complex mix of lipids, including fatty acids. However, for ethical, economical and scalability reasons, serum-free and defined media for cultivated meat and seafood are essential. A major challenge lies in replicating the beneficial lipid environment of FBS-containing media, while reducing cost and complexity. This study focuses on identifying an effective strategy for formulating customized lipid mixtures tailored to the specific needs of Oncorhynchus mykiss (Rainbow trout) cells in suspension culture. First, the fatty acid profiles of standard FBS- and/or commercial lipid mixtures-containing media were characterized using mass spectrometry. Key fatty acids were identified, quantified, and ranked for both their abundance and economic viability. In addition, delivery methods such as detergents and bovine serum albumin were tested to assess bioavailability and cellular uptake of the fatty acids. Several of these lipid/carrier mixtures were tested in reduced serum or serum-free media formulations and evaluated for proliferation and fatty acid consumption of fish cells. Some of these lipid mixtures successfully replicated the performance of FBS-containing media in a defined manner. Moreover, cell assays indicated that not all fatty acids are required for proliferation, which enabled a considerable simplification of lipid mixtures. The resulting defined lipid mixture supports healthy cell proliferation and offers a scalable, cost-effective alternative to serum-derived lipids for cultivated fish applications.

Abstract # 76

Membrane-Based Recovery of Growth Factors from Cell Culture Spent Media for Sustainable Cultivated Meat Production

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The high cost of cell culture media, accounting for 30–40% of total expenses in cultivated meat production, is a major challenge1. Around 70% of media cost is due to Fetal Bovine Serum (FBS), which provides essential growth factors.2 Media is changed every 2–3 days to avoid the accumulation of toxic metabolic waste products, such as, lactate and ammonia, both of which inhibit cell growth.3 The spent media retains over 50% of its nutrients, including proteins and growth factors, but is often overlooked due to lack of efficient downstream processing.4

This study explored the use of 10 kDa ultrafiltration regenerated cellulose membranes to selectively recover proteins and growth factors from spent media, enabling their recycling in cultivated meat production. Bradford assay results show that ultrafiltration membranes can recover 97% of the total protein content. The recovered protein is added to basal media to create recycled media. SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) analysis shows that the protein composition in the recovered media matches the size of those in fresh media. Cedex BioAnalyzer measurements indicate that inhibitor concentrations in the recovered media are negligible. The C2C12S cell line was used to verify the bioactivity of recovered proteins. Cell viability, morphology, and growth rates showed no significant differences between cells cultured in recovered and fresh FBS media for at least three passages. These findings demonstrate the potential of ultrafiltration-based media recycling as a sustainable and cost-effective strategy for cultivated meat production.

Abstract # 77

Changes in bovine satellite cell culture media over time elucidated by 1H NMR spectroscopy

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The culture media is essential for maintaining a tightly controlled environment that supports optimal growth of cells for cultivated meat production. However, frequent media change is resource-intensive and economically unsustainable for large-scale production systems. To optimize media usage and reduce operational costs, it is crucial to understand how the composition of culture media changes over time to identify limiting nutrients and accumulation of potentially cytotoxic compounds. Therefore, the aim of the present study was to investigate metabolic changes occurring in the culture media over time. For this purpose, bovine muscle satellite cells from three bull calves were cultured in 48-well plates for 7 days. Culture media was collected on days 1, 2, 3, 4, 5, and 7. Cell counts were performed using Hoechst staining, and media samples were analyzed using 1H NMR spectroscopy. Analysis of spent culture media revealed a marked increase in lactic acid concentration over time, while glucose levels remained stable. Quantification of amino acids revealed divergent trends, with certain amino acids including alanine and glutamic acid increasing in concentration, while others showed a decline. The findings indicate that lactic acid accumulation may contribute to reduced media performance over time, despite sufficient glucose availability. The declining concentrations of certain amino acids may indicate limitations in their availability over time. Understanding these changes provides a foundation for possible optimization of media composition.

Abstract # 80

Assessment of 'complex ingredients' as media components for the development of cost-effective animal component-free culture media

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Expensive and unsustainable animal-derived ingredients, such as fetal bovine serum (FBS), remain a key barrier to the commercial viability of cultivated meat. Plant- and microbial-based extracts (complex ingredients) may serve as functional, cost-effective alternatives. Numerous business-to-business (B2B) companies now offer plant- and microbial-protein ingredients that provide nutritional and bioactive molecules with the potential to support cell proliferation.

We investigated the use of these ingredients as media components to replace animal-derived inputs and develop cost-effective media formulations (single £/L) that match the performance of traditional serum-containing media. A landscape review identified 56 candidate ingredients across five categories: plant protein isolates, plant protein concentrates, plant hydrolysates, yeast extracts, and non-yeast microbial biomass.

We evaluated these ingredients in porcine adipose derived stem cell (ADSCs) culture using one-factor-at-a-time (OFAT) assays and artificial intelligence (AI)-led media optimisation. Twenty ingredients promoted growth and maintained low precipitation over 60 hours in OFAT testing, while none exhibited cytotoxicity. We included 11 high-performing complex ingredients in a multi-iteration media optimisation campaign alongside 20 defined ingredients (e.g. amino acids, vitamins). After three optimisation rounds, testing a total of 1,020 formulations, 412 reached over 70% of the growth seen in 10% FBS media, with 190 of those achieving a cost of goods below £10/L.

Our proprietary ingredient selection algorithm consistently identified six complex ingredients as key contributors to performance and cost-effectiveness. This work demonstrates that B2B complex ingredients, when combined with advanced AI tools for ingredient selection and formulation optimisation, can enable the development of commercially viable animal component-free media.

Abstract # 82

Whey as cell growth supplement in cultivated meat production

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Milk whey is one of the earliest dairy by-products, shown to promote the growth of various mammalian cell types. Milk contains three vital elements of cell culture media as growth factors, adhesion factors, and transport proteins. Presently we demonstrated whey proteins (WPs) as a sustainable alternative to FBS in cultivation of C2C12 muscle cells, for application in cultivated meat production. Cells were cultured in media with WP mixture (β-lactoglobulin 0.07%, α-lactalbumin 0.15%, bovine serum albumin 0.15%), 10% FBS (positive control), or without (negative control) for 48h (day-1 and -2) to support proliferation. Subsequently, cells were switched to low-mitogenic 2% horse serum medium until full differentiation (day-6). Both WPs and FBS media significantly enhanced cell viability and proliferation (XTT assay, microscopic analysis), while maintaining cell membrane integrity (lactate dehydrogenase assay) on day-2 compared to untreated cells ($p<0.05$). On day-6, these cells differentiated into myotubes and expressed myogenesis-specific markers, creatine kinase and citrate synthase ($p<0.05$). Also, RT-qPCR analysis showed increased expressions of myogenesis-specific genes (MYOG, DES, MRF4, MYH2) compared to untreated cells ($p<0.05$). Furthermore, preliminary proteomics analysis revealed WPs to positively regulation cellular pathways corresponding to myogenesis.

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Abstract # 83

Development of a Serum Alternative for Cell Culture by Reconstructing Organ Crosstalk In Vitro

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Serum, typically derived from animal blood, has long been a key component in cell culture media due to its ability to support cell growth. However, its use can be associated with certain limitations, such as variability between batches, potential contamination risks, ethical considerations, and relatively high costs. Therefore, the development of a defined, animal-free alternative that more closely mimics physiological conditions is of growing interest. Here, we propose reconstructing organ crosstalk in vitro as a novel strategy for developing such a serum alternative.

We developed a co-culture system named CulNet (Culture Network) comprising primary cells and cell lines derived from chicken embryos. The system consists of combinations of cells derived from three different organs selected from ten organ types: brain, lung, heart, stomach, liver, kidney, intestine, muscle, skin, and membranes (chorioallantoic, yolk sac, amnion). Conditioned media collected from this optimized multi-organ co-culture system, termed CulNet serum, underwent comprehensive proteomic and metabolomic analyses, revealing bioactive factors crucial for cell growth and homeostasis.

The efficacy of CulNet serum was evaluated against traditional fetal bovine serum using primary cultured cells derived from duck embryo liver, chicken embryo muscle tissues, and chicken cell lines. The results demonstrated equivalent or superior cell viability and proliferation compared to serum-supplemented controls.

Furthermore, scalability and batch-to-batch consistency were confirmed, demonstrating compatibility with large-scale bioreactors. This innovative serum alternative significantly advances cellular agriculture, particularly cultivated meat production, with additional applications in regenerative medicine and tissue engineering by enhancing reproducibility, ethical standards, and cost efficiency.

In conclusion, reconstructing organ crosstalk in vitro successfully yielded a defined, effective, and physiologically relevant serum alternative, representing a critical step toward sustainable and reliable cell culture systems.

Abstract # 84

Optimise the light spectrum to improve the growth and nutrition of microalgae for the sustainable production of cultured meat.

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Considering increasing global huge demand of resources and energy, as well as ethical concerns such as animal welfare, it is a comprehensive search is essential to maximize the benefits of untapped bioresources.

Cell-cultured meat technology, which potentially meets the global protein demand by mass-amplifying parts of somatic cells without sacrificing animals, has attracted attention as a meat production way that replaces conventional livestock meat to, and research has been progressing.

In this situation, we are developing a sustainable cell culture for producing cultured meat by replacing grain-derived ingredients with microalgae-derived thereof.

A previous study has already been conducted on the utilisation of degraded microalgae in animal cell cultures.

In anticipation of transitioning from a laboratory to an industrial scale, this study examined microalgae culture condition, such as wavelength, intensity, and duration of the light spectrum, to maximize the effectiveness for animal cell culture.

We evaluated it using microalgae growth and examination of nutrients extracted from them.

It is known that certain species of microalgae, including Haematococcus lacustris (Girod-Chantrans) Rostafinski, exhibit a propensity to thrive under specific light spectra.

In this study, the light spectrum and intensity were varied to demonstrate the optimal growth conditions for freshwater microalgae, including Anabaena (Trichormus sp. PCC 7120a: capable of nitrogen fixation) and Chlorella vulgaris (NIES-2170: used as health food and supplements).

Abstract # 85

Fungal and Algal Extracts Enrich Myoblast and Mammary Cell Growth in Serum-Free Culture Medium

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Fungal and microalgae extracts represent a sustainable strategy to enrich cell culture media for cellular agriculture, providing bioactive molecules that may support cell adhesion, differentiation, and 3D-matrix development. This study evaluated aqueous extracts from Pleurotus ostreatus (PL), PL grown on peptone (PLP), Mucor spp. (M), Saccharomyces cerevisiae (Y), Euglena gracilis (EG) and whey (W) for their effects on murine myoblasts (C2C12) and bovine mammary epithelial cells (MTEC). Standard culture medium, without fetal bovine serum (FBS), was supplemented with extracts at 3.13-0.02 mg/mL. Samples were chemically characterized (dry matter, ash, crude protein, crude fiber, ether extract). Cell viability was assessed using Alamar Blue assay after 48 hours (C2C12) and 72 hours (MTEC). The three most effective concentrations for each extract were used to qualitatively evaluate morphology and proliferation by inverted light microscopy and to monitor dynamic cell proliferation using xCELLigence system. In parallel, RT-qPCR was performed to analyze genes involved in proliferation (in both cell line) and differentiation (in C2C12). Protein contents were 16.54±0.70% for PL, 42.41±0.76 for PLP, 42.22±0.48% for M, 46.59±0.43% for Y, 29.47±0.12% for EG, 78.80±0.18% for W. PL extract promoted C2C12 proliferation at 0.78 mg/ml and showed the highest MTEC viability at 1.56 mg/mL, although other concentrations also supported cell viability. PL, PLP, M showed higher max Cell Index than cells grown on only FBS, indicating enhanced cell proliferation. These results support the use of fungal and microalgae extracts to promote cell growth in serum-free media.

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Abstract # 87

Antibiotic-free fed-batch strategy for cultivated fish cells: From Lab to 50 L Scale

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Objective:
Demand for seafood is expected to increase significantly by 2050 due to population growth, rising incomes, and changing dietary habits. However, the sustainability of this demand is called into question by the current state of wild fish stocks, of which approximately 90% are fully exploited, overexploited or depleted. Cultivated seafood offers a sustainable alternative, but scalable, food-grade bioprocesses are required. This study presents a 50 L production process for antibiotic-free rainbow trout cells, focusing on feeding strategies to maximise biomass yield.

Methods:
A fed-batch cultivation process was developed at laboratory scale, incorporating nutrient feed to increase the yield. This strategy was scaled up to 50 L using power input per volume (P/V). The process was conducted fully antibiotic-free to simulate future production environments.

Results:
The core feeding strategy, particularly the nutrient feed, was successfully transferred to the 50 L scale, effectively increasing biomass output by 50 %. Key process characteristics, such as growth dynamics and yield trends, were successfully replicated.

Conclusion:
This work demonstrates that a fed-batch strategy optimised at laboratory scale can be effectively transferred to an antibiotic-free 50 L process. The successful application of a nutrient boost highlights its value in enhancing yields, even under scaled-up conditions. These findings support the development of robust, scalable bioprocesses for cultivated seafood and emphasise the potential of process strategies to maintain performance across production scales.

Abstract # 88

Developing a perfusion process to maximize cell yield

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Background:
Cultivated seafood offers a healthy and sustainable alternative for worldwide fish stocks, which are already overexploited and increasingly contaminated. One major step towards bringing cultivated seafood to the market is to develop a food-grade bioprocess operating at high productivity.

Methods:
In this study, a perfusion process was investigated to increase the yield of cultivating fish cells at lab-scale: Utilizing cell retention in stirred-tank bioreactors, exhausted medium was exchanged and replenished based on cell-specific perfusion rates. In-vessel filtration was used for size-based cell retention. Alternating flow was applied to extend run time and maintain perfusion rates.

Results:
Operating a perfusion process increases cell yield while maintaining high cell viability and morphological characteristics. In-vessel cell retention with alternating flow achieved 100% cell retention. Process optimization, including automation, resulted in a stable and repeatable process. Further intensification by repeated fed-batch extended process runtime and additionally boosted productivity.

Conclusions:
This study shows productivity can be increased drastically by process intensification. Further development of the bioprocess, including optimizing the applied media, will be crucial in maximizing productivity to deliver cultivated fish at scale for the market.

Abstract # 90

Comparative environmental impact assessment of culture media of the scaled-up Circular Cell Culture system: media based on algae Chlorella Vulgaris, cyanobacteria Anabaena, and DMEM

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Production of culture media ingredients contributes heavily to the environmental impacts of cultivated meat. Alternative sources of nutrients for culture media must both support cell proliferation and reduce the environmental burden when produced on a large scale.

This study assesses the environmental sustainability of culture media in the scaled-up Circular Cell Culture (CCC) system. The CCC uses nitrogen fixing cyanobacteria or algae as sources of nutrients. The environmental impacts of culture media based on algae Chlorella Vulgaris, cyanobacteria Anabaena, and traditionally used Dulbecco's Modified Eagle Medium (DMEM) are compared. Comparisons are done based on the efficiency in supporting the growth of QM7 quail muscle cells in the CCC.

The production systems that include algae/cyanobacteria cultivation, nutrient extraction, and subsequent culture media use are scaled-up to the industrial level in SuperPro Designer. The cradle-to-gate prospective life cycle assessment (LCA) study is conducted following ISO 14040/ISO 14044 standards. The inventory is modelled based on the experimental data from the laboratory, literature, and databases.

Compared to DMEM, AE and CVE require more electricity use, with hotspots in cultivation and nutrient extraction processes. Electricity use causes most of the environmental impacts of AE- and CVE-based culture media. Hence, their comparison with DMEM-based medium depends highly on the electricity sources.

This work helps quantifying the prospective environmental performance of scaled-up cultivated meat using culture media derived from circulated nutrients. This project is a collaboration between the University of Helsinki, Finland, and Tokyo Women's Medical University-Waseda University Joint Institution for Advanced Biomedical Sciences (TWIns), Japan.

Abstract # 91

Microbial lysates as cost-effective substitutes in cell culture media

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A significant barrier to the production and commercialization of cultivated meat is the cost of crucial media additives such as amino acids and recombinant proteins. Our group has previously demonstrated that microbial protein lysates can act as low-cost replacements for fetal bovine serum in cell culture media. In the present study, we are now investigating using these lysates to replace amino acids and growth factors in culture media. We have successfully expressed fibroblast growth factor 2 (FGF-2) in Vibrio natriegens, a fast-growing marine bacterium. Subsequently, we have demonstrated that BSCs (bovine stem cells) grown on media supplemented with V. natriegens FGF-2 lysate achieve similar growth rates as BSCs grown on recombinant FGF2-supplemented media. We are continuing to investigate the growth of other cell types such as bovine de-differentiated fat cells (BDFATs) on our V. natriegens FGF-2 lysate-supplemented media. Additionally, we are exploring the use of our microbial lysates to replace amino acids in culture media. Here, we employ commercially available enzymes, Alcalase and Flavourzyme, to break down protein lysates from Kazakhstania humilis and Vibrio natriegens into their constitutive peptides. We have developed and optimized an enzymatic hydrolysis protocol and have qualified an assay that quantitatively measures the degree of hydrolysis. We have measured a 4x increase in hydrolysis between enzymatically treated lysates and untreated lysates. We are currently testing the growth of BSCs grown on culture media supplemented with our microbial-derived peptides rather than commercially available amino acids.

Abstract # 92

Empirical Life Cycle Assessment of Cultured Meat Production in Japan: Current Impacts and Improvement Pathways

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Several studies have been conducted on the environmental impact assessment of cultured meat production. However, as cultured meat is an emerging technology, many of these previous studies are primarily predictive, and the environmental impacts of current production processes are not well understood. Recently, regulatory approval processes for the commercial sale of cultured meat have progressed in various countries. As a result, the predominant production scale among cultured meat companies worldwide is currently at the pilot scale, which is suitable for correcting the data required for safety assessments. With the expected transition to commercial-scale production following regulatory approvals, the industry is currently in a transitional phase. Once a commercial scale facility is constructed, it will be difficult to make major changes or redesign. Therefore, it is essential to assess the environmental impacts of current cultured meat production and identify challenges and improvements in order to establish a low environmental impact production system. This study quantitatively assessed the environmental impacts of current cultured meat production using life cycle assessment based on actual production data. The primary data for this analysis were provided by Integriculture Inc., Japan. This study compares the results with the environmental impact of livestock meat production. Furthermore, based on these results, we explore strategies for establishing low-impact cultured meat production systems and identify key challenges and improvement directions. The findings of this research will contribute to the establishment of low environmental impact cultured meat production system and the social implementation of cultured meat.

Abstract # 95

Genetic disruption of CDKN2A delivers a robust and precise method for the generation of immortalized bovine satellite cell lines

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Commercial production of cultivated meat requires a steady supply of cells that can reproducibly proliferate and differentiate into muscle tissue, even after extended culture. While primary bovine muscle progenitor cells lose their proliferation rate and differentiation capacity over time, immortalized cell lines could offer a virtually unlimited source of cells, creating paths to rapid commercialization. Here, we report the first evidence of a precise, simple, and reproducible single gene knockout strategy that yields functionally immortalized bovine satellite cells (iSCLs) through CRISPR-Cas9 based genome engineering. We show that disruption of the cell cycle related gene CDKN2A is sufficient to immortalize two genetically distinct primary bovine satellite cell strains. iSCLs replicated over 75 times in culture with a stable 17h doubling time while retaining their differentiation capacity in 2D and 3D systems. Compared to their respective primary cell strains, iSCLs showed robust Ki-67 staining along with minimal SA-β-galactosidase activity across extended culture, suggesting an escape from senescence. Additionally, iSCLs proved more stress-resistant in unfavourable growth conditions, such as low confluency and low growth factor culture medium. Finally, we show this strategy works with a dual Cas9 D10A nickase mRNA-based expression approach, thereby reducing off-target effects and avoiding the insertion of foreign DNA into the genome, a regulatory advantage compared to overexpression systems. By combining functional immortality with regulatory compatibility, this strategy provides a reliable method for generating gene-edited immortalized bovine satellite cell lines, minimizing batch variability and enabling scalable cell production for cultivated meat.

Abstract # 100

Fish cell expansion in bioreactor: assessing expansion and process intensification

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Cultivated seafood production relies on efficient fish cell expansion systems that allow to scale such process. Besides, fish muscle cell differentiation leads to the development of mature muscle fibers, potentially enhancing the nutritional and sensorial quality of cultured fish products. In this study, the DASbox® Mini Bioreactor System was used to optimize Dicentrarchus labrax embryonic cells (DLEC) and Scomber scombrus myoblast (MACK1) expansion. Different edible and porous HybridX and potato microcarriers (provided by Gelatex) were used as platforms for cell adherence and growth. The process was optimized by assessing growth kinetics, population doubling time, and cell viability at different operating conditions. After that, MACK cells was characterized by immunostaining (for myosin heavy chain) and RT-PCR (for MYOD1, MYOG, PAX3B, and TNNT3A). Finally, we combined the 3D microcarriers loaded with fish cells with edible algae- and plant-based materials to prepare bioinks and to 3D bio-print a complex cultivated fish product. The cell viability and morphology after the 3D bioprinting process was characterized by Live/Dead assay.

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Abstract # 103

CELL CULTURE MEDIA COMPONENTS FOR CULTIVATED MEAT – How WACKER Enables Manufacturing of Cultivated Meat

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The use of expensive growth factors and serum replacements poses a significant barrier to the widespread adoption and commercialization of cultured meat. In the present study, we investigated whether the food additive, cyclodextrin, in different grades and purities is suitable for use in the production of cultured fat and cultured meat.

Cyclodextrins, due to their unique molecular structure, have shown potential as media additives to enhance cell proliferation in serum-free conditions, a critical challenge in the commercialization of cultured meat. Our study investigated the effects of various cyclodextrins, including CAVAMAX® W6 (alpha-cyclodextrin) and W8 (gamma-cyclodextrin) Food non-GMO, CAVASOL® W7 M (methyl-beta-cyclodextrin) and HP (hydroxypropyl-beta-cyclodextrin) on the proliferation of multiple cell types relevant to cultured meat production. Concentrations ranging from 0.125 mM to 10 mM were applied in combination with growth factors, such as FGF-2 and BSA, revealing even stronger effects on cell proliferation. We thereby prove the ability of cyclodextrins to optimize media composition and enhance cell viability and proliferation. These findings highlight the potential of cyclodextrins to improve cell culture conditions in serum-free environments, offering a cost-effective and scalable solution for cultured meat production. Further research is warranted to elucidate the mechanisms underlying the differential effects of cyclodextrins across various cell types and concentrations. This study provides a foundation for the development of chemically defined media tailored to the needs of diverse cell types in the cultured meat industry.