PAT to Optimize the Cost, Consistency, and Yield of Cultivated Meat Production

White Paper

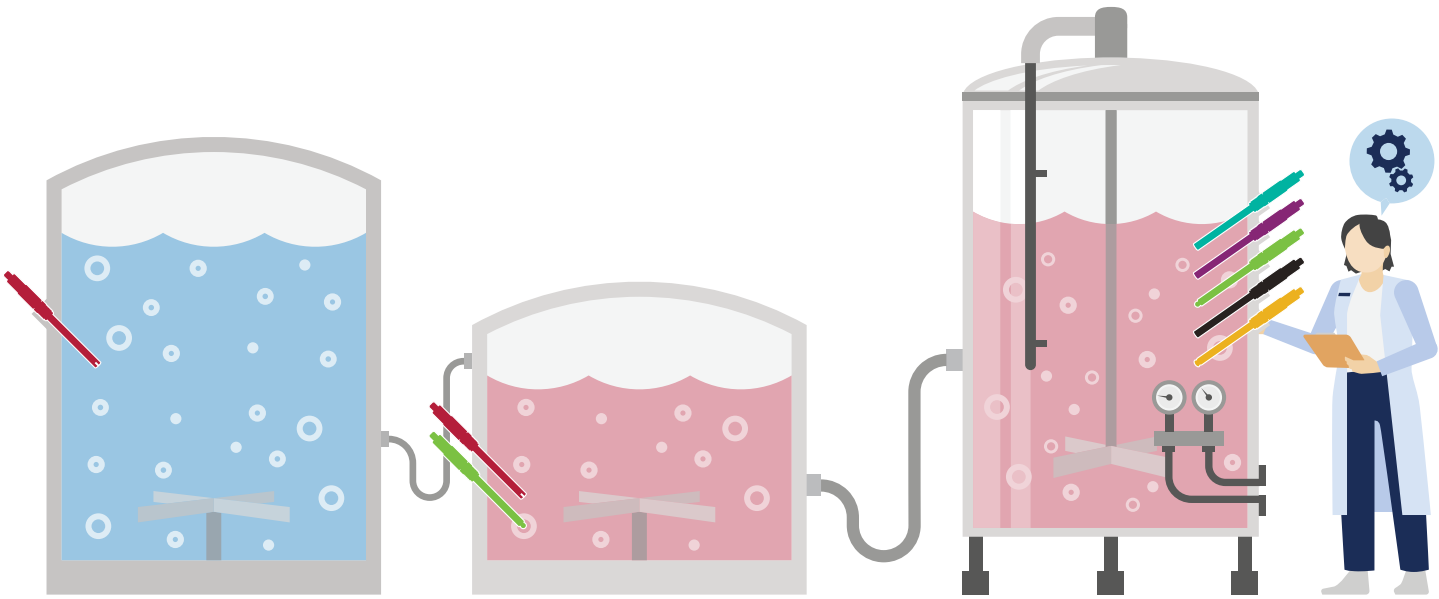
Table of Contents

Introduction

By 2050 the global population is expected to approach 9-10 billion[1] leading to an expected doubling of the global food demand[2]. As food demand continues to rise, new technologies and food production processes will become increasingly necessary to increase the production of high-value proteins, while simultaneously reducing their environmental footprint. “New” foods, including cultivated meat produced through cellular agricultural processes, could be one solution to address the challenge of sustainably supplying sufficient, nutritious protein sources to future generations.

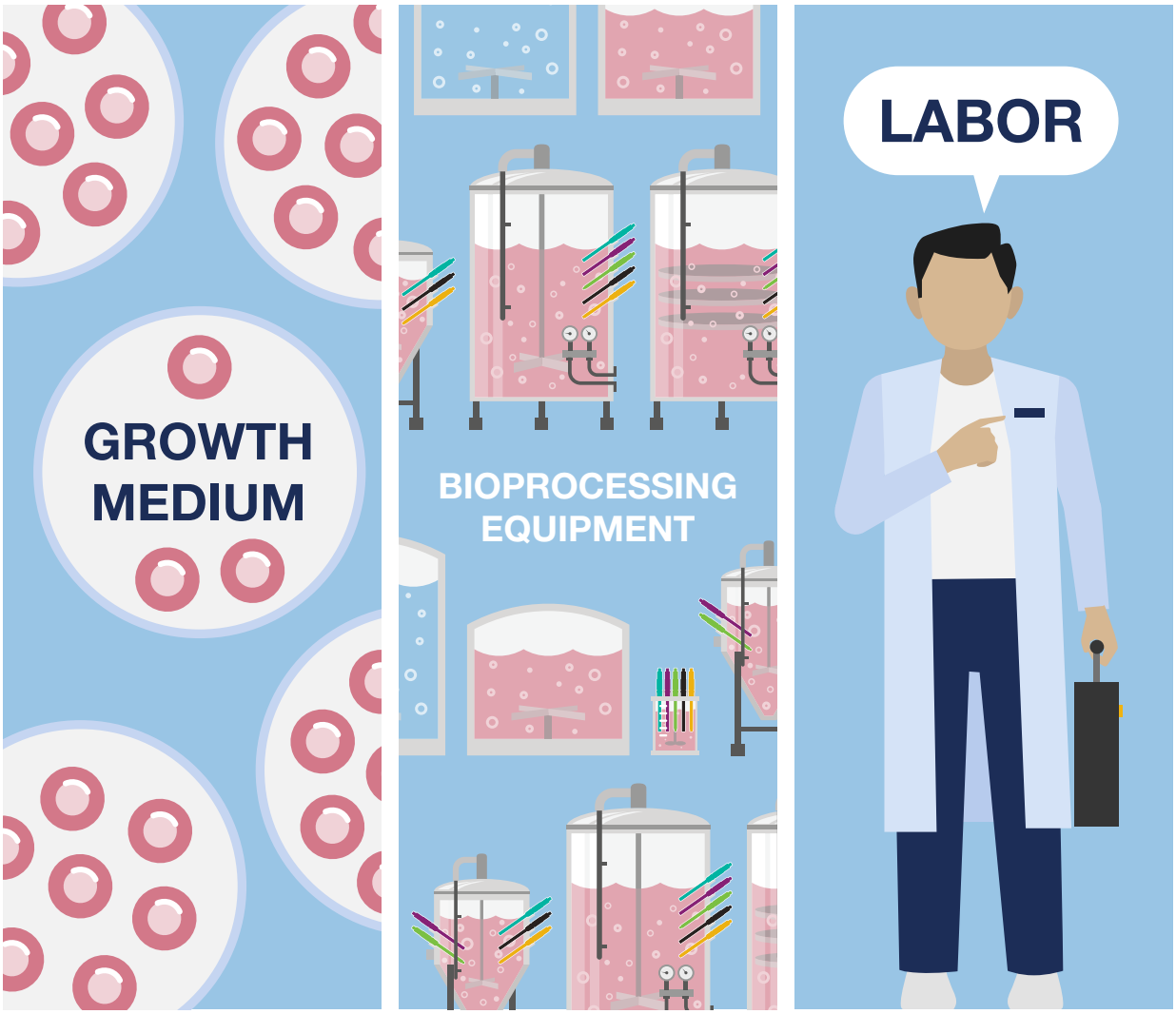
Although "New foods" present many benefits and fulfill many of these concerns, they are faced with challenges: financing, R&D, regulatory processes, and consumer acceptance. Currently, there is an appetite for investing in cultivated foods[3] and enduring the regulatory processes that vary in timelines between regions and countries[4]. At the time of writing, two companies have been approved in Singapore and the US, however high-production costs limit the affordability of cultivated meats for consumers. As a process reliant on reproducibly producing high-quality, viable-cell-dense cultures in large volumes, their biggest challenge is reliably and efficiently optimizing production processes during scaling. It takes about 2.9 x 10^{11} muscle cells to produce one kilogram (2.2 pounds) of cultured meat[5], in a process which takes two to eight weeks to complete[6] at a predicted cost of $63/kg[6]. This raises the important question: how can the cultivated meat industry cost-effectively optimize their processes to improve consistency and yield during production?

At Hamilton Process Analytics, our expertise lies in the application of innovative sensor technologies to cell culture challenges. As we partner with some of the top innovative companies in the sector, we developed this white paper to outline common technical challenges faced by the cultivated meat industry, alongside case-study examples from complementary cell culture applications to demonstrate how some of these challenges could be overcome through process optimization procedures aiming to cost-effectively improve the consistency and yield of cultivated meat production.



1. Challenges in the Cultivated Meat Industry

The process of cultivated meat production can generally be divided into two main phases: (1) establishing solid protocols for cultivated meat production and (2) scaling processes for commercial production. For both phases, the main technical cost drivers limiting production success are (1) growth medium, (2) bioprocessing equipment and (3) labor[6]; with additional challenges including cell lines, scaffold (and microcarrier) materials and production protocols, increasing biomass yield and how to scale production, and end product considerations including taste, texture and smell for both raw and cooked products. The principles of many cell-culture processes aiming to produce large volumes of high-density cell cultures are readily applicable to the cultivated meats industry[7]; therefore, it is not illogical to conclude that process controls could be similarly adopted by this emerging industry to improve process performance. Process Analytical Technologies (PAT) are tools that could be used to measure and control critical parameters and key process indicators during cultivated meat production processes in real-time to ensure high-quality, high volume yields are achieved during process optimization.



Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

2. PAT in the Context of Cultivated Meat Production

Process Analytical Technology tools are encouraged by the U.S. Food and Drug Administration (FDA) and other regulatory bodies for food manufacturers to optimize the efficiency, reproducibility and reliability of their production processes[8]. By using PAT throughout production processes, manufacturers gain a better understanding of their processes, enabling improved control and regulation of critical process parameters for and monitoring of key process indicators for improved consistency in attributes in the final product. PAT can therefore be key to accelerating process optimization by reducing timelines for commercialization and can be used as gateway tools for process documentation in preparation for submission to regulatory bodies.

For the cultivated meat industry, implementation of PAT can help to build a complete, accurate picture of the processes occurring in bioreactors, tanks, and other apparatus, and could ease the transitions between milestones in a products lifetime (e.g., during scale-up from R&D to large-scale production), and overall improve the reliability, reproducibility, and cost-efficiency of processes during production. Utilizing control measures early in the production workflow, as far upstream as possible, can help to minimize if not alleviate downstream problems, increasing the reliability of end products having the desired critical quality attributes.

Definition of PAT

PAT Fundamentals

PAT are frameworks for innovation as they introduce checkpoints during production that can be used to control and regulate specific properties of a product (e.g., taste, texture, nutrients). This is achieved through a Quality-by-Design (QbD) approach: where quality checks are built into every step of the manufacturing process, rather than an inefficient, potentially costly post-production quality testing approach. Quality is built into a product using (1) knowledge of the product itself, and (2) understanding how every component of the production process can influence the quality of the final product. To establish a QbD protocol, the following variables must be defined:

- Critical Quality Attribute (CQA): a physical, chemical, or biological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

  E.g., taste, protein content, fatty acid composition, etc.

- Critical Process Parameter (CPP): a process parameter whose variability has an impact on a critical quality attribute and, therefore, should be monitored or controlled to ensure the process obtains the desired quality.

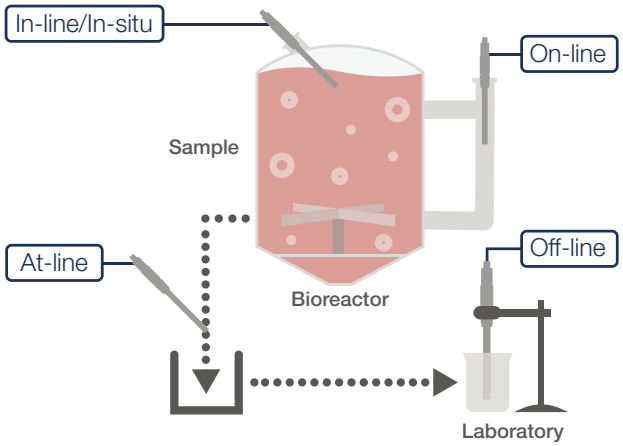
  E.g., pH, dissolved CO_2, dissolved O_2, temperature, etc.

- Key Performance Indicator (KPI): a metric for the status of each production step. KPIs are related to CQAs and therefore influenced, as well, by the CPPs. As the CPPs remain within the pre-defined limits, the KPIs should indicate the relative success of each production step and the likelihood of the CQAs being present in the end product.

  E.g., cell growth and density

PAT Measuring Methods

Process analyzers can be implemented in different positions along production (Figure 1). The differences between these methods in the context of the bioreactor will be the focus of this white paper, and will be discussed below.



Process Desirables	PAT Method			
	Off-line	At-line	On-line	In-line
Direct Measurement			✓	✓
Real-time Analysis			✓	✓
Automation Compatible			–	✓
Cost-efficient Monitoring Method	✓	✓	–	✓
Simple to Implement	✓	✓	–	–

Figure 1: (Left) Placements of the methods to apply PAT to monitor processes at the bioreactor, according to PAT guidance (2004). (Right) Comparison of the delivering process desirables for each PAT method. (✓) indicates process desirable is attributed to the method. (-) indicates process desirable is achievable, with some effort or process modification.

In-line (and to an extent, on-line) measurements are the preferred choice as they offer direct monitoring of processes in real-time for data-driven adjustment of critical process parameters. Although they may incur higher costs initially (compared to at-line and off-line measurements), this is quickly off-set by the reduced running costs when we consider fewer down-times (and associated loss of expensive resources and products) due to continuous, direct monitoring of processes. And when we think about the implementation of automation (only possible with in-line and modified on-line measurements), further cost reductions are possible due to the redundancy of personnel and manual handling and analysis, in addition to increasing efficiency of processes by reducing the frequency of process delays.

Including automatic in-line controls in cultivated food production processes, as opposed to off-line controls, can lead to significant cost savings and waste reduction, including:

1. **Efficient Quality Control:** In-line control systems can continually monitor production parameters, ensuring that the product quality is maintained throughout the production process, reducing the need for end-of-line inspections which can be costly and time-consuming.
2. **Waste Reduction:** By monitoring production in real time, in-line control systems can quickly identify and rectify issues, reducing the amount of product that is wasted. For example, integrating in-line measurement instrumentation can reduce hold times and the risk of off-spec product, aiming for waste reduction to less than 1% as compared to common figures of 5% or more seen in conventional setups.
3. **Increased Throughput and Plant Availability:** In-line controls can greatly improve production check steps, increasing throughput and reducing hold times. This translates to higher plant availability and potentially more revenue generation.
4. **Real-Time Adjustments:** Automatic in-line controls allow for real-time adjustments to the production process based on the data collected, thereby reducing the likelihood of errors, and minimizing waste caused by production changeovers, recipe errors, or production delays leading to raw material degradation.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

5. **Resource Optimization:** Effective cost control, facilitated by in-line controls, prevents waste, and helps in maintaining healthy profit margins. This could be achieved through optimal utilization of raw materials, energy, and labor.

6. **Automation and Technological Advancements:** Engaging automation and advancements like neuro-fuzzy controllers, as a part of in-line control, can lead to optimal food process control, furthering the cause of cost savings and waste reduction.

The exact cost savings and waste reduction figures would depend on the specifics of the technology implemented, the scale of operations, and other contextual factors within the food production facility.

Technologies for Process Analytics

There is a variety of different technologies that can be used for process analytics, the suitability of a particular technology for a specific process and analyte (critical process parameter) will depend on (1) sampling method, (2) sample preparation, duration of analysis, and process application.

Broadly, PAT tools can be categorized into the following:

- Multivariate tools for process design, data acquisition and analysis.
- Process analyzers (e.g., in-line sensors or automated at-line devices).
- Process control tools (e.g., statistical process control software).
- Continuous improvement and knowledge management tools.

Common process controls include:

- In-line control of fundamental critical parameters for cell culture technologies: pH and Dissolved Oxygen, followed by in-line monitoring of other CPPs such as total and viable cell density and dissolved CO_2.
- Off-line gas analyzers monitoring the in-and off-gas composition as indicators of cellular respiration.
- In-line metabolite analysis (e.g., glucose and lactate) for information on the biochemical status and lifecycle of cells.
- Off-line spectroscopic methods (e.g., Raman, nuclear magnetic resonance) to determine the presence of secreted components during the first stage of media conditioning.

Additional secondary process analyzers based on off-line molecular spectroscopy techniques such as Raman or Near-Infrared spectroscopy may be useful for the cultivated meat industry, however it is worth noting the space, expense, expertise, personnel and time required for implementation of these techniques may outweigh their usefulness while navigating the initial stages for this emerging sector, therefore will not be reviewed here.

PAT in Food Applications

Implementation of PAT in food manufacturing has been practiced in several areas of food production under Process Analytical Chemistry[9,10], where samples represent complex mixtures of heterogeneous molecules (proteins, fats, carbohydrates) often on complicated matrices (amorphous solids, aqueous liquids, gels, macromolecules, macro-organelles, cells, crystal sizes, pore sizes, etc.) that can make process monitoring more complex, yet more important. Although complex, monitoring of processes is essential in the food industry to ensure customer safety when consuming foods, therefore adoption of PAT has served as a risk analysis strategy.

Moving towards process monitoring technologies with real-time capabilities such as in-line sensors, allows industries to move from retrospective quality control towards continuous measurement of core quality parameters enabling active control of processes. In the food industry, PAT has helped to move away from inefficient and potentially costly "post-process" or "feed-back" control where the end product is tested and the process adjusted accordingly, towards "during-problem" or "model-predictive" control where large variations in raw materials are considered and used to adjust processes to achieve specific qualities in the end product[10,11].

Extensive examples of the various technologies and applications of PAT in the food industry are available in the literature including cheesemaking[12,13], confirmation of food provenance[14,15], food degradation and quality analyses[16] and contamination [7].

Aim: achieve a safe end-product containing the desired quality attributes in a cost effective, traceable, and environmentally responsible way.

PAT in Cell Culture Applications

In cell culture applications, PAT is used as part of a quality-by-design (QbD) approach aiming to improve the efficiency of a biomanufacturing process by ensuring end products reliably contain pre-determined desirable attributes[18,19]. Through a QbD approach, the effect of parameters on process performance and productivity can be monitored by measuring key performance indicators. Generally, the bioreactor is central for sampling and measurement of aqueous cell suspensions[20]. Here, in-line, on-line, at-line and off-line tools can be used to measure cell viability, apoptosis and aggregation; off-gas composition, nutrient and metabolite concentration as indicators of cell growth and health[20]. In-line sensors open the possibility of integration with control systems for feedback-driven approaches during process monitoring of critical parameters for advanced bioprocess control[21,22]. Hamilton offer a range of in-line sensor solutions for measuring parameters including dissolved CO_2 (CO_2NTROL), dssolved oxygen (VisiFerm RS485), pH (EasyFerm Bio) in addition to key process indicators including viable cell density (Incyte Arc) and total cell density (Dencytee Arc) for cell culture applications.

Technological advances have seen the advent of intelligent sensors through integration of wireless technology directly in the sensor that allows the sensor to talk directly to the PCS without the need for a transmitter, sending compensated measurement values and diagnostic data, the latter of which is also automatically stored on the sensor itself. Through Arc technology, communication between Arc-enabled sensors and the PCS can be configured to and monitored with a computer or mobile device for flexibility and convenience. For example, the Incyte Arc capacitance sensor (Hamilton) is an intelligent capacitance (or permittivity) sensor used to measure the viable cell density (VCD) of processes using a measurement methods that can only measure intact (living) cells and is unaffected by the presence of cell debris or dead cells. This differs from Dencytee Arc, the total cell density sensor (TCD), which is an optical density (turbidity) sensor that indiscriminately measures the transmittance and reflectance of near-infrared light of particles and molecules (living cells, dead cells, debris) in the pathlength during both low-and high-density applications, respectively. Both viable-and total-cell density are important key process indicators with can signal the success and performance of a process by signaling that conditions during bioprocessing were optimized sufficiently to support cell growth and proliferation.

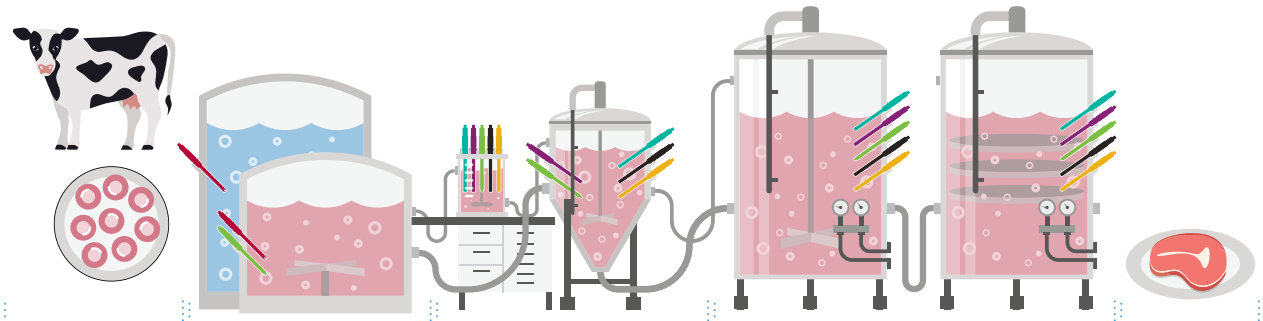
Aim: achieve desired end-product quality in a cost effective and traceable way.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

The Potential of PAT in Cultivated Meat Production

The cultivated meat industry lies at the intersection of cell culture and food science therefore PAT tools and approaches have applications in this novel multi-disciplinary industry. The industry's reliance on the production of large volumes of high cell-density cultures suggests the transferability of knowledge from related industries [7] and the potential benefits for adopting similar process controls by this emerging sector.

Aim: enable the cost effective, traceable, and sustainable production of safe, edible products with high levels of pre-determined desirable quality attributes.



		1 Cell Line Derivation	2 Media & Water Prep	3 Bioprocess: Proliferation	4 Differentiation/ Maturation	5 Final Product
On-line	pH		×	×	×	×
	COND		×			
	TCD			×	×	×
	VCD			×	×	×
	CO_2			×	×	×
	DO			×	×	×
Off-line	Biomolecular and Molecular Spectroscopy Methods	×	×	×	×	×
	Microscopy Methods	×		×	×	×

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

Below, suggestions for PAT tools at each stage of cultivated meat production are listed.

1

Cell Line Derivation

A starting cell line is created with stem cells and muscle cells non-invasively harvested from live animals e.g., from embryos, biopsies, or iPSC.

Attributes to monitor using PAT:
Media composition, cell viability, nutritional profile.

In-line Sensor:

- pH – EasyFerm Bio – ensuring optimum pH maintained in culture medium for cell growth. pH can be influenced by the concentrations of dissolved CO_2 in the media, therefore feedback actions via pH adjustment in addition to gaseous exchange (sparging, mixing, in-gas composition) can be implemented.
- Dissolved oxygen – VisiFerm RS485 – sufficient O_2 bioavailability for aerobic respiration is essential for cell survival, and can be regulated via sparging and stirring rates in addition to composition of the in-gas.
- Dissolved CO_2 – CO_2NTROL – gaseous exchange and CO_2 removal to prevent hypoxia and medium acidification. Measurement alongside O_2 provides insights into respiration processes in cultures and can be used as a complementary measurement for pH regulation when connected to pH adjustment, stirring and sparging controls.
- Total cell density – Dencytee Arc – determination of total cell accumulation.
- Viable cell density – Incyte Arc – determination of viable cells after harvesting as an indicator of their fitness for cell culture applications. Can also be used as a feedback initiative for the effectiveness of the parameter regulation, e.g., the impact of impeller speed on cell viability.

Off-line spectroscopy and microscopy methods: Near Infra-Red Spectroscopy, Mass Spectrometry, Acoustic Resonance Spectroscopy, Calorimetry, Dielectric Spectroscopy, Fluorescence Spectroscopy, High-Performance Liquid Chromatography, In-Situ Microscopy, UV Absorbance.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

2

Proliferation

Harvested cells are expanded through a seed train before being introduced into the primary bioreactor.

Attributes to monitor using PAT:
Media composition, cell viability, cell metabolism.

In-line Sensor:

- pH – EasyFerm Bio – ensuring optimum pH maintained in culture medium for cell growth. pH adjustment can be controlled by addition of base in addition to sparging and mixing rate.
- Dissolved oxygen – VisiFerm RS485 – sufficient bioavailability of O_2 for aerobic respiration during the stage where cell density is exponentially increasing can be controlled by sparging and mixing rate in addition to in-gas composition.
- Dissolved CO_2 – CO_2NTROL – gaseous exchange and CO_2 removal to prevent apoptosis, especially important when considering the exponentially increasing number of cells. It is key in bicarbonate buffer pH adjustment. It is a critical parameter during scale-up of processes, as important as pH and dissolved O_2.
- Total cell density – Dencytee Arc – determination of total cell accumulation.
- Viable cell density – Incyte Arc – determination of viable cells accumulated throughout the proliferation stage as a measure of the compatibility of the physical (e.g., impeller speed) and chemical (e.g., pH) cultivation conditions in addition to feeding strategies for cell survival and growth. In cultivated food processes viable cell density is a direct measurement of the productivity.

Off-line spectroscopy and microscopy methods: Near Infra-Red Spectroscopy, Raman Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, Mass Spectrometry, Dielectric Spectroscopy, Fluorescence Spectroscopy, High-Performance Liquid Chromatography, In-Situ Microscopy, Terahertz Technology, Photoacoustic Spectroscopy, UV Absorbance.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

3

Differentiation/Maturation

Once optimum cell density is reached, cells are encouraged to differentiate into the three main components of meat: muscle, fat, and connective tissue through a combination of media components (e.g., addition of growth factors facilitating myogenesis)[23–26] and adhesion to microcarriers and/or scaffolds[23] (depending on the cell type, process and structural complexity of the end product).

Attributes to monitor using PAT:
Media composition, cell viability, cell metabolism, cell attachment, cell thickness.

In-line Sensor:

- pH – EasyFerm Bio – ensuring optimum pH maintained in culture medium for cell growth. pH adjustment can be controlled by addition of base in addition to sparging, mixing and perfusion flow rate of media.
- Dissolved oxygen – VisiFerm RS485 – sufficient bioavailability of O_2 for aerobic respiration during stages where cell density is very high and cell are likely adhered to a physical support (microcarrier and/or scaffold). In-gas composition, sparging, mixing and perfusion flow rate can be used to regulate dissolve oxygen concentrations.
- Dissolved CO_2 – CO_2NTROL – gaseous exchange and CO_2 removal to prevent hypoxia and medium acidification, especially important when considering the close proximity an potentially fixed position of cells while adhered to microcarriers and/or scaffold. Correct CO_2 control ensures the lactate-shift happens in cells to ensure viability. Sparging, mixing and perfusion flow rate can be used to regulate dissolve oxygen concentrations.
- Total cell density – Dencytee Arc – determination of total cell accumulation.
- Viable cell density – Incyte Arc – determination of viable cells during differentiation and maturation as an indication of the potential end product yield and quality and can aid understanding of the effects of specific parameters on product quality. The ratio between viable cell density and total cell density constitutes the viability which can be an important parameter to control.

Off-line spectroscopy and microscopy methods: Near Infra-Red Spectroscopy, Raman Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, Mass Spectrometry, Dielectric Spectroscopy, Fluorescence Spectroscopy, High-Performance Liquid Chromatography, In-Situ Microscopy, Terahertz Technology, Photoacoustic Spectroscopy, UV Absorbance.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

4

Final Product

Cells have continued to grow and adhere and have now reached a density sufficient to form the final product, ready for consumption. Qualities like nutrient content can be analyzed to feedback into the production design to optimize nutritional content of the final product.

Attributes to monitor using PAT:
Media composition, cell viability, nutritional profile, cell thickness.

In-line Sensor:

- pH – EasyFerm Bio – pH is an important determinant of meat freshness, taste and quality.
- Dissolved oxygen – VisiFerm RS485 – now that the end product is ready for consumption, oxygen exposure will need to be restricted to improve shelf-life by reducing or delaying oxidation and microbial spoilage. Optimum oxygen concentrations to extend shelf-life and to improve or at least maintain the taste, appearance and quality of the product.
- Dissolved CO_2 – CO_2NTROL – CO_2 is both water- and lipid-soluble, increasingly so with decreasing temperature. Dissolution of CO_2 reduces the local pH which (1) reduces respiration of cells in the product and (2) has a bacteriostatic effect, which conjointly extend the shelf-life of the product. However, high dissolution of CO_2 into the product can affect product taste and texture. Determination of optimum CO_2 concentration in the end products modified atmospheric packaging should be determined to ensure shelf-life and quality of the end product.
- Total cell density – Dencytee Arc – determination of total cell accumulation.
- Viable cell density – Incyte Arc – determination of viable cells in the final product as an indication of quality and shelf-life. VCT measurements can provide inshight into the effectiveness of controlling other parameters on the end product.

Off-line spectroscopy and microscopy methods: Near Infra-Red Spectroscopy, Raman Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, Mass Spectrometry, Acoustic Resonance Spectroscopy, Calorimetry, Dielectric Spectroscopy, Fluorescence Spectroscopy, High-Performance Liquid Chromatography, Microwave Resonance Technology, Photoacoustic Spectroscopy, UV Absorbance.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

As evidenced from the previous steps, **PAT can be applied throughout all stages of cultivated meat production** to address the maintenance of some key attributes in the final product. At Hamilton, we recommend the implementation of in-line sensors:

Incyte Arc – For direct measurement of viable cell density as an indicator of produced meat mass.

CO_2NTROL – A low-maintenance solid-state (glass-free, food-grade stainless steel) optical dissolved CO_2 sensor for regulation of both CO_2 concentration and pH.

VisiFerm RS485 – A low-maintenance solid-state (glass-free, food-grade stainless steel) optical dissolved oxygen sensor.

EasyFerm Bio – pH sensor designed for food applications containing a biocompatible Foodlyte electrolyte.

Furthermore, implementation of automated feedback and reactions controls can help with improved process control and efficiency, in addition to reducing the need for manual labor and associated costs.

Having established the usefulness of PAT during production, the next step is to **identify the key challenges** for the industry as a starting point to **determine where intervention could alleviate these challenges**.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

3. Optimized Cost, Consistency and Yield in Cultivated Meat

As highlighted previously, the main technical cost drivers limiting production are (1) growth medium, (2) bioprocess equipment and (3) labor. Having identified areas during cultivated meat production where PAT could be implemented, this white paper will continue to examine how the implementation of PAT could be used to address these technical cost drivers limiting the commercialization of cultivated meat production.

Media Optimization

Culture media is essential to sustain cells during manufacturing processes, and according to the Good Food Institute, the costs of creating cell culture media will make up 55% to 95% of marginal costs of cultivated food production[27], therefore optimization of media formulations could reduce the cost burden of this essential production component. Furthermore, optimized media formulations could improve the efficiency of cell growth and differentiation, reducing time and physical resources for processes, further improving the economic feasibility of production. Protocols for obtaining media of specific nutrient and growth factor composition vary and are under development by various groups in the sector, as described below. During longer-term, continuous culture methods (fed-batch, perfusion) feeding strategies (in addition to media formulation,) also influence the bioavailability of the life-sustaining nutrients to cells and can directly affect productivity.

Media Formulations

At its most basic essence, basal cell culture medium provides a carbohydrate-energy source (normally glucose), inorganic salts, water-soluble vitamins, amino acids as its ingredients; though the proportions of each vary depending on cell-line and application and whether cells are growth in vivo and ex vivo[28–31] therefore requires optimization for each process, application, and cell type. Furthermore, media composition can affect not only cell growth and health, but also quality attributes of the cells and therefore the final product such as nutritional profile.

Additional factors (including recombinant proteins unsustainable, and uneconomical[27,32,33,27,32,33], and are often dependent on stimulatory or inhibitory effect[34,35], such as Incyte Arc could be used to monitor changes in cell-growth behaviors as indicators for limited growth-factor availability during processes. Research into alternative factor formulations include platelet-serum[36,37], single protein candidates e.g., sericin[38–41], serum-free[2,43], food industry by-products[30], algae-based[44] and co-culture approaches[45]) are underway and have been adopted by some companies within the industry e.g., Mosa Meats[43], Omeat. Modelling approaches could help to fine tune media formulations of the future[46], and further research into reducing costs for food-grade reagents and reliable technologies is required. Most analytical techniques for medium metabolite composition rely on technically-and labor-intensive at-line or off-line instrumentation such as High-throughput LC-MS or Raman spectroscopy[47]. For these spectroscopic techniques, the development of in-line technologies would be useful for the cultivated meat industry as they could be used during medium optimization protocols, especially during metabolomic analysis during media conditioning[48,49]. For example, Incyte Arc could be used to monitor changes in cell-growth behaviors as indicators for limited growth-factor availability during processes. Research into alternative factor formulations include platelet-serum , single protein candidates e.g., sericin, serum-free, food industry by-products, algae-based and co-culture approaches) are underway and have been adopted by some companies

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

within the industry e.g., Mosa Meats, Omeat. Modelling approaches could help to fine tune media formulations of the future, and further research into reducing costs for food-grade reagents and reliable technologies is required. Most analytical techniques for medium metabolite composition rely on technology- and labor-intensive at-line or off-line instrumentation such as High-throughput LC-MS or RAMAN spectroscopy. For the cultivated meat industry, rather than these technology-and labor-intensive spectroscopic techniques, the development of in-line technologies can be used during medium optimization protocols, especially during metabolomic analysis during media conditioning.

Conditioned Media

Conditioned Media describes cell culture media following its use in cell-growth processes[50–52] and is one method that can be used to minimize costs associated with the purchase of expensive growth factors: primary cell cultures could be selected to recombinantly produce specific growth factor or favorable growth conditions for cultivating cells for meat products. Nevertheless, the cost-reward for multiple rounds of cultivation of different cells should be considered.

Furthermore, the roles of specific growth factors and cytokines (chemical-signaling molecules produced by cells) on cells-of-interest, and the secretome of cells used to produce conditioned media and in subsequent cultures should be known to allow for the optimum. Some cultures can secrete growth-factors in a self-sustaining manner[53], and co-culture of different cells may be a viable way to achieve economic longevity of processes[45] in addition to preventing contamination and growing cells in an antibiotic-free environment[4,54]. Furthermore, conditioned media has applications in the alternative protein sector using fermentation methods with microbes to produce edible biomolecules[55] which could further increase novel food productivity from both sectors.

Recycling Media

This refers specifically to the implementation of recycling technologies aimed at capturing and reusing costly media components (e.g., growth factors, recombinant proteins)[53,56]. Compared to traditional agriculture, some novel foods could be more sustainable (energy, wastewater, land use, emissions)[57]. The potential of applying such technologies to cultivated meat production would reduce costs of expensive media ingredients and conditioning processes.

Feeding Strategies

To achieve maximum cell outputs, optimal growth rates are essential. In addition to extraneous environmental control (e.g., pH, oxygen availability), cells fundamentally require sufficient nutrient supply. During cell culture applications, cultures can be grown using fixed volume (and therefore nutrient) or variations of longer-term variable volume (batch-fed or perfusion) methods that utilized a feeding strategy. Feeding strategy management can be used to ensure maximal cell growth while minimizing costs associated with expensive media formulations and waste management: overfeeding leads to nutrient excess and waste accumulation, while underfeeding can result in nutrient and energy-source depletion inducing cell stress and reduced cell growth.

CASE STUDY

Potential to Maximize Cultivated Meat Productivity through VCD In-situ Measurement

This study investigated the effect of nutrient availability on cell growth by implementing a control loop that regulated the pump rate of media in response to viable biomass determined using Hamilton's capacitance probe, Incyte Arc. When measured on-line in 6-second intervals, rapid feedback on changes in growth rate in response to growth conditions enables data-driven adjustment of parameters in bioprocesses to maximize performance (Figure 2).

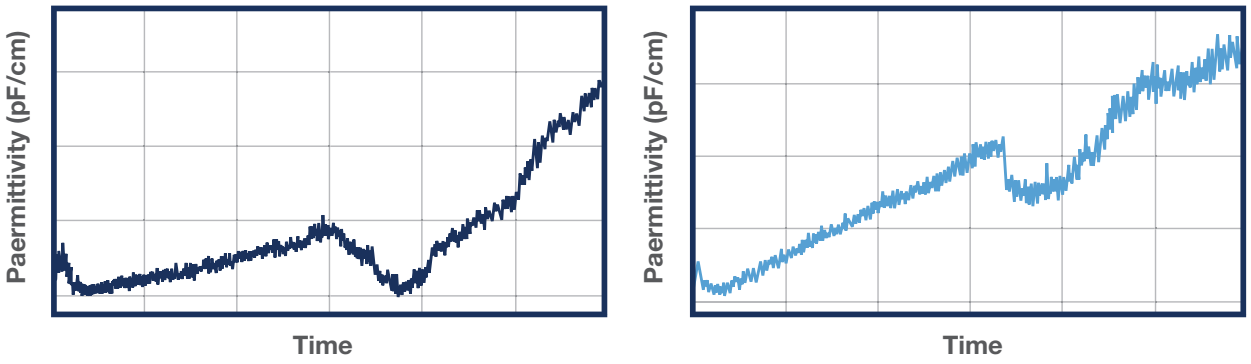


Figure 2: Incyte Arc sensor permittivity measurement with insights into process comparison. Run 2 on the right side shows an increased efficiency due to the optimized process parameters.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

Process Optimization

Before commercialization is achieved, processes must be optimized to determine the ideal parameters for optimal cell growth during each stage cultivated meat production. This requires (1) having a deep understanding of processes and a clear quality by design approach, (2) identifying and forecasting potential bottlenecks for contingency planning and (3) acknowledging the scale stages are not equal (i.e., R&D ≠ pilot ≠ commercial) therefore must be optimized independently. Starting from the cell lines used and their behavior at each stage, to reagent and formulations, and process design and instrumentation, every step must be considered.

Bioreactor and Process Design

For most bioprocesses, there are generally three separate phases: lab-scale, pilot-scale, and demonstration or commercial scale. Bioreactors (also known as cultivators) are housing vessels which enable the careful control of conditions for optimum cell growth and are broadly categorized as batch, fed-batch, continuous, and perfusion[58–60], and are used throughout all phases. In batch culture, a fixed volume of media is used to grow cells to maximum density before moving onto the next step (e.g., harvesting or transfer to a larger vessel). Fed-batch culture differs in that fresh media is supplemented in-line to cells at variable rates to maximize a specific process indicator such as exponential cell growth or cell density of the culture. In continuous culture, media is continuously exchanged via in-line feed and collection channels using independently optimized flow-rates, while cells are collected separately. Perfusion culture, a subset of continuous culture, recycles medium while retaining cells in the vessel, permitting higher cell densities to be achieved in a smaller space. There are benefits and challenges for each culture technique, therefore multiple methods are commonly used throughout a process.

Within the bioreactor, the interactions between cells (solid phase), culture media (liquid phase) and headspace (gas phase), although complex, are important variables for consideration, and can be investigated using physical (e.g., temperature), chemical (e.g., pH: EasyFerm RS485, DO: Visiferm Arc) and biological parameters and process indicators [e.g., cell concentration: Incyte Arc and Dencytee Arc]. Other factors affecting process performance include gas exchange, heat transfer, nutrient homogeneity, pH homogeneity, shear stress, mixing, and foaming. Integration of state-of-the-art sensor equipment into the bioreactors themselves during the planning phase, alongside developing processes that are amenable to automation, medium recycling, and waste valorization will overall maximize the efficiency of a process and ensure sterile, food-grade production environments are maintained.

CASE STUDY

Importance of Measuring the Interaction Between Parameters O_2, pH and CO_2

This study looked at the influence of O_2, pH and CO_2 on viable cell density over the course of long-duration processing. Measuring O_2 and CO_2 can give insights into the respiration of cells, which can be extrapolated as indicators of cell viability which can be supported by other measurements including off-line cell counting (as was performed in this study) or in-line capacitance measurements (e.g., Incyte Arc, Hamilton) which offer the benefits of real-time feedback on processes compared to off-line measurements.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

In this study, pO_2, pCO_2 and pH set points were set and maintained throughout the fermentation process using a novel control strategy due to the need to independently monitor each parameter. Normally in cell culture, pCO_2 is considered an acid therefore is included in pH control. For this experiment, pH was instead measured in-line using an EasyFerm pH sensor (Hamilton) and regulated independent of pCO_2 using HCl/NaOH; pCO_2 was measured by an off-gas sensor; and pO_2 was measured in-line using a VisiFerm DO sensor (Hamilton).

Their results showed that visible cell density is affected by pH – especially when considering longer processing time, and pH had the largest impact on process durability out of the three parameters studied (Figure 3). Therefore, determining the pH for your processes should be the first step during optimization to ensure improved process performance and longevity. This study was performed using batch culture, therefore the implications in perfusion should be considered.

Furthermore, pCO_2 was measured using an off-gas PAT tool, which provides 1-dimensional data due to the lack of information on the pCO_2 in the media, which will have a greater impact on cell biology and process performance due to its proximity to cells. Our suggestion, as PAT experts, for comprehensive information on the effect of specific parameters during processes, is the implementation of in-line measurement PAT tools. In the case of dissolved CO_2, Hamilton's CO_2NTROL is a solid-state optical dissolved CO_2 sensor, which continuously measures dissolved CO_2 concentrations in real-time and benefits from the direct optical measuring method (compared to traditional indirect Severinghaus measurement methods). This study decoupled pH regulation from dissolved CO_2 concentration for the purpose of investigating the impact of the individual parameters, but during standard cultivated meat production, measuring dissolved CO_2 is an powerful multi-functional tool for monitoring changes in concentrations of dissolved respiratory gases as indicators of cell viability in addition to pH adjustment when considering the acidity of dissolved CO_2. CO_2NTROL can therefore be considered a food-safe, glass-free tool for monitoring pH during cultivated meat production. This study clearly demonstrates how PAT tools are key to measuring and understanding the interactions of multiple parameters such as pH, O_2 and CO_2 in bioprocesses.

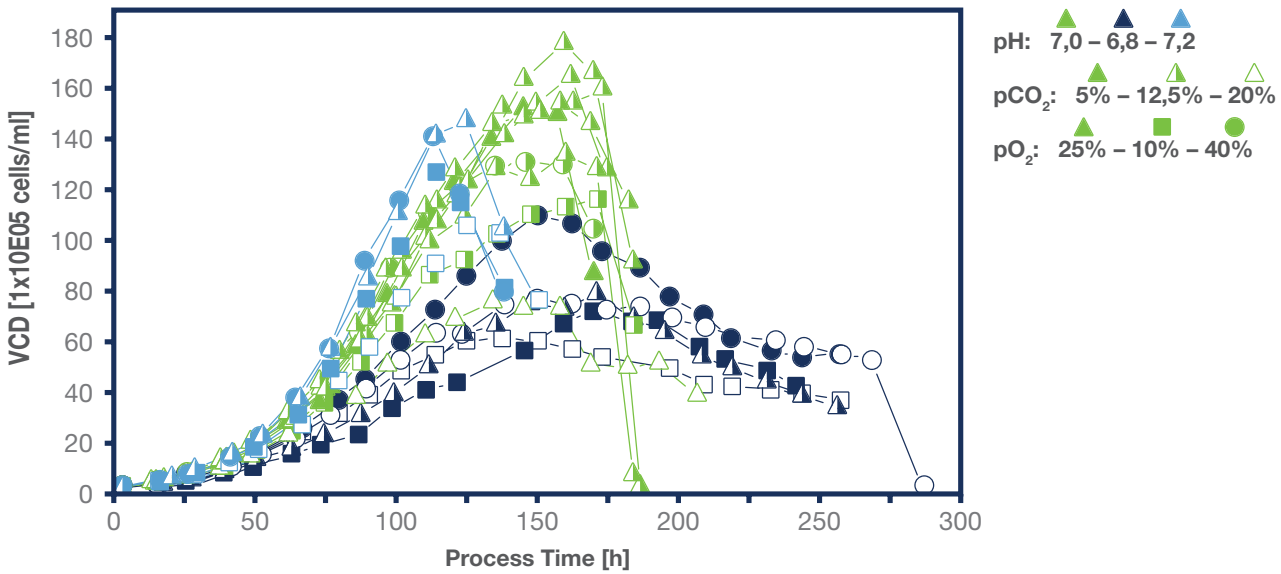


Figure 3: Viable cell density over process time for all batch fermentations. (Green symbols represent processes at pH 7.0, dark blue symbols at pH 6.8, light blue symbols at pH 7.2; closed symbols represent processes at pCO_2 5%, half-closed at 12.5% and open symbols at 20%; triangles represent processes at pO_2 25%, squares at 10%, circles at 40%). High pH values led to high viable cell densities but concurrently to shorter process time due to faster depletion of the main c-source. Cell viabilities stayed at high values as long as glutamine was available. Figure adapted from[61].

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

CASE STUDY

The Importance of Determining the Optimum pH Control Strategy for Each Process

Large-scale bioreactors are economic solutions when scaling-up production, however simple volumetric comparisons minimize the real-time effects of process parameters on performance and output. Generally, larger bioreactor volumes require longer mixing times, and inefficient strategies can result in pH gradients and inhomogeneous environments which can negatively affect process performance.

This study focused on pH, an essential parameter for cell health and viability, and the impact of different regulation strategies on the viability of two different cell lines. Here, they demonstrated that the addition of base, regardless of strategy, is a stressor for cells (indicated by the high cell viability in the absence or postponement of base (Figure 4), but when regulating pH (EasyFerm, Hamilton), tight and consistent control is comparatively favorable when considering cell viability. This study also highlights the need to consider the specific cell-line used when optimizing processes (Figure 4) to minimize negative process performance.

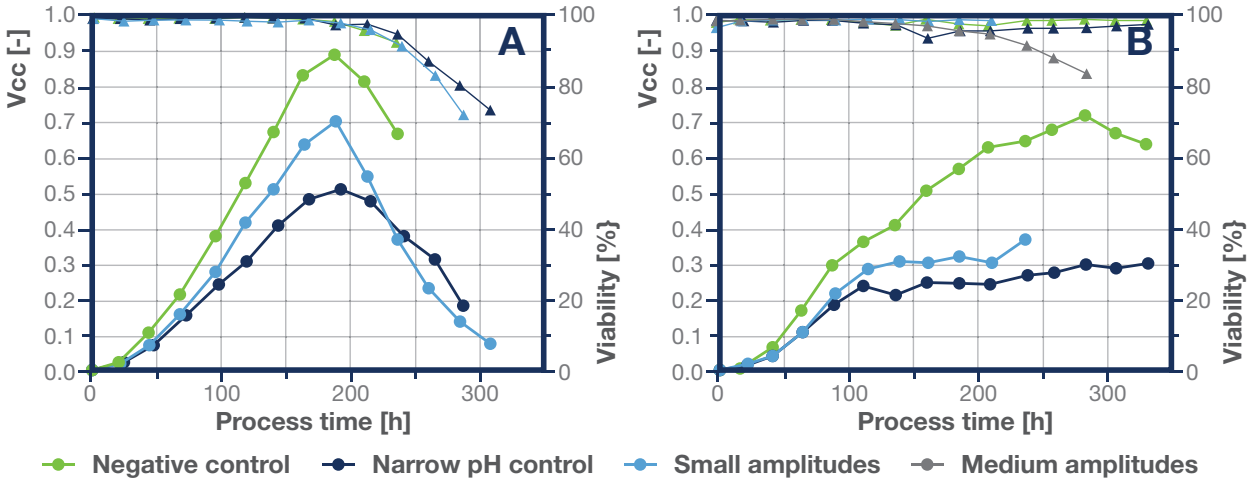


Figure 4: Cell viability for different cell lines when the pH of culture medium is regulated using different methods. pH was measured using Hamilton's EasyFerm pH sensor in-line, and was regulated using either a negative control (pH regulation stopped after 180 h), narrow pH control (no pH amplitude), or small amplitude (±0.05 pH units) approach. (A-B) Viable cell concentration (dots) and viability (triangles) trajectories are shown. Dissolved oxygen was measured using Hamilton's VisiFerm DO sensor. Figure adapted from[62].

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

CASE STUDY

Elevated pCO_2 Affects the Lactate Metabolic Shift in Mammalian Cell Culture Processes

The metabolic shift from lactate production to consumption is a key event during cell culture, reportedly triggered by reductions in environmental pH (often related to elevated CO_2 concentrations in older cultivations) and the availability of substrates like glucose and glutamine. It is therefore often associated with the adaptation of cells to a depleting nutrient availability after long cultivation periods, as they adapt to increase cell longevity and survive and is seen as a desirable process in cell culture.

This study showed the lactate metabolic shift was absent in batch and fed-batch cultures at elevated pCO_2 when compared to cultures at lower pCO_2 values (Figure 5). Metabolic analysis indicated the metabolic shift is triggered by a reduction in the oxidative capacity of cells in high CO_2 environments, which is of particular interest during large-scale and perfusion processes due to the likelihood of the accumulation of CO_2 over long processes and highlight the need for measurement and control of CO_2 as a critical process parameter during cell culture.

During this study CO_2 was measured using off-gas analyzers which provide information on the composition of gas in the headspace of the bioreactor as indicators of cellular respiration. However, no information is provided for the concentration of dissolved CO_2 in the media – the primary location for the accumulation of aerobic respiration by-products produced by cells suspended in culture media during bioprocesses. Considering the importance of the results with respect to longevity and productivity of cultivation processes, this only highlights the need for manufacturers to (1) consider CO_2 as a critical process parameter and (2) to monitor CO_2 using in-line sensors such as Hamilton's optical dissolved CO_2 sensor, CO_2NTROL (Hamilton) for real-time feedback on processes and cell performance.

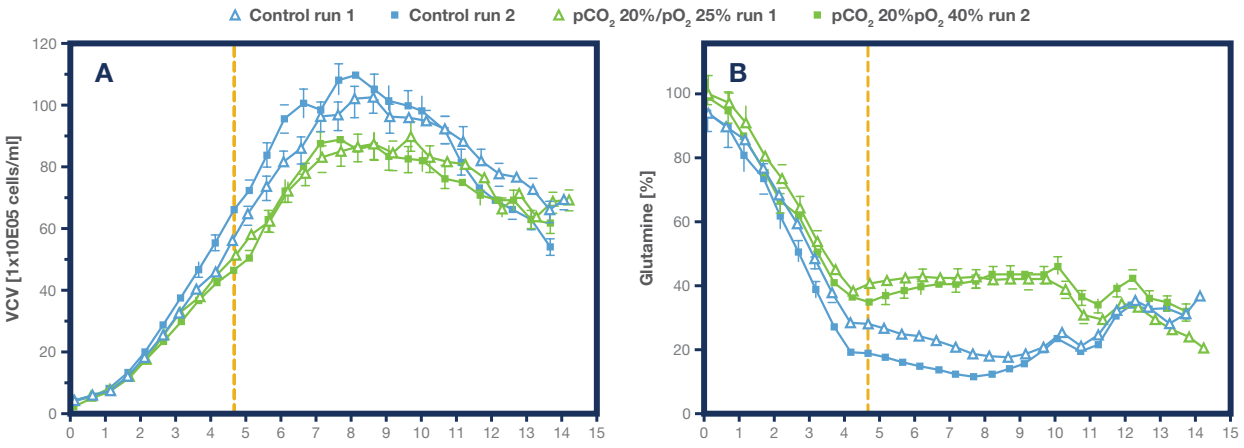


Figure 5: Viable cell density (VCD) and metabolite concentrations over process time of the fed-batch fermentation processes. Cultures at pCO_2 20% did not switch from lactate production to consumption, in contrast to the control cultures at 12.5% pCO_2. No depletion of glucose or glutamine occurred during the processes. The time of the metabolic shift is indicated by the dashed line. pH was instead measured in-line using an EasyFerm pH sensor (Hamilton) and regulated independent of pCO_2 using HCl/NaOH; pCO_2 was measured by an off-gas sensor; and pO_2 was measured in-line using a VisiFerm DO sensor (Hamilton). Error bars indicate range of analytical accuracy of measurements. Figure adapted from[63].

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

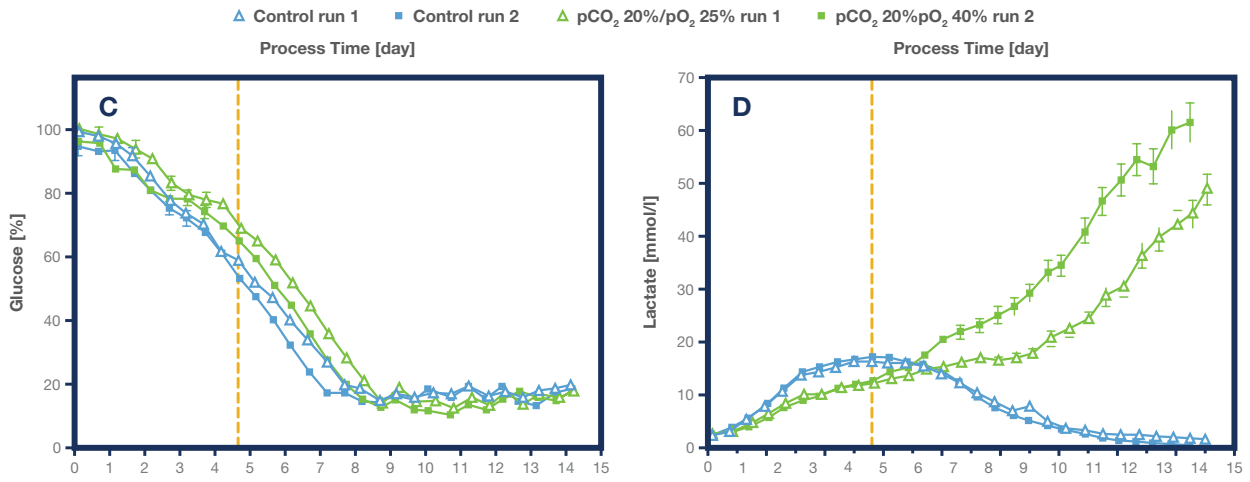


Figure 5: Viable cell density (VCD) and metabolite concentrations over process time of the fed-batch fermentation processes. Cultures at pCO_2 20% did not switch from lactate production to consumption, in contrast to the control cultures at 12.5% pCO_2. No depletion of glucose or glutamine occurred during the processes. The time of the metabolic shift is indicated by the dashed line. pH was instead measured in-line using an EasyFerm pH sensor (Hamilton) and regulated independent of pCO_2 using HCl/NaOH; pCO_2 was measured by an off-gas sensor; and pO_2 was measured in-line using a VisiFerm DO sensor (Hamilton). Error bars indicate range of analytical accuracy of measurements. Figure adapted from[63].

PAT in Scaling

PAT is key for reliable scaling. Currently, a significant challenge to producing cost-effective cultivated meat and seafood is the scalability of production. Individual bioreactors have a limited capacity, while manufacturers have limited facility space. Producers are therefore presented with the dilemma of (1) to scale-up or scale-out, and (2) what cultivation method to use (batch, fed-batch, continuous, or perfusion). Compared to scale-out processes, where multiple units of the same volume vessel are used, scale-up involves increasing the volume of the culture vessel and consideration of variables including gas exchange, heat transfer, nutrient homogeneity, pH homogeneity, shear stress, mixing, and foaming in the bioreactor. Technological advances have seen the emergence of hybrid systems which are more efficient in resource and utility consumption and can produce large volumes of high-density cultures in relatively smaller footprint facilities. Seed trains are cell-growth strategies for progressive up-scaling of cell cultures and rely on the transference of cells to larger vessels at the correct window when the highest density of cells is exponentially doubling in population. This is due to the influence of (1) the volume and density of viable cells in the inoculation culture, and (2) the volume of the growth vessel will influence the processing time.

Regardless of approach, a key interest during scaling is to economically obtain the maximum number of cells from each run. Adoption of PAT during scaling can address some of the concerns regarding the potentially negative aspects of the increasing culture volume during up-scaling.

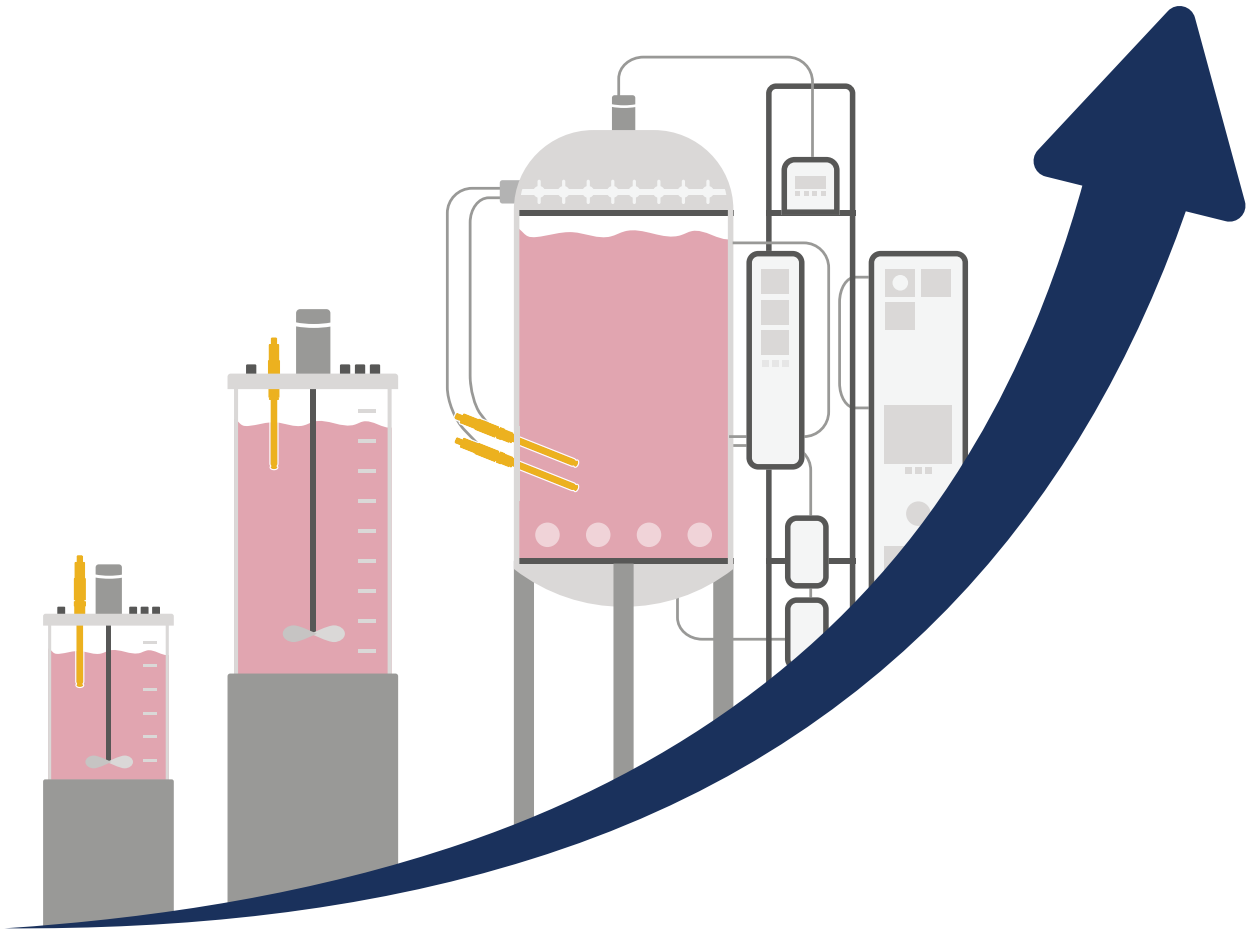
Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

CASE STUDY

More Efficient Scale Transfer

This study investigated an optimization strategy using N-1 perfusion cell culture. During seed train intensification (N-1) stage (before the production bioreactor, N), culturing techniques move from fed-batch to perfusion with the aim of intensifying seeding densities during N-1 production. By intensifying viable cell density in the N-1 volume before the production bioreactor and using an in-line probe to determine the proportion of viable cells in a given volume, this ensures production processes will have the maximum potential to quickly adjust and establish in larger volumes during scaling-up protocols.

In this study, capacitance was measured using Hamilton's Incyte Arc viable cell sensor. Incyte Arc is an intelligent sensor which benefits from ArcAir capabilities including sensor self-diagnostics, wireless communication between sensor and control center, in addition to data modelling for better process insights, such as better determination of timings for culture transfer during scaling. Measurements are collected in real time, empowering the user to determine when the viable cell density threshold for transference has been reached in the N-1 stage.



Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

During high-density culture techniques, a major concern for production is ensuring high volumes of viable (living) cells are produced, especially during scaling procedures as this enables maximum recovery rates when cells are transferred to the production bioreactor. This study tested the congruity between off-line cell density measurements and in-line Incyte Arc biocapacitance measurements across 6 different cell lines and multiple scales (from 5L to 500 L) (Figure 6), and showed agreement between the two measurement methods. When comparing both methods, one key benefit is highlighted for in-line over off-line methods: the continuous measurement of in-line methods offer superior process control relative to discrete off-line methods. This is because in-line methods have shorter measurement windows (6 seconds for in-line Incyte Arc compared to 24 hours for off-line measurements), making it easier to identify the best time for scale transfer. This study demonstrates the usefulness of capacitance measurements when optimizing the timings for seed-transfer during upscaling procedures, especially important during proliferation stages of cultivated meat production. For each bioreactor volume, the minimal viable cell density requirements for efficient cell growth after scale-transference can be calculated based on the rates of recovery of cell cultures in the new bioreactor volume after transfer, empowering users to familiarize and better understand their processes.

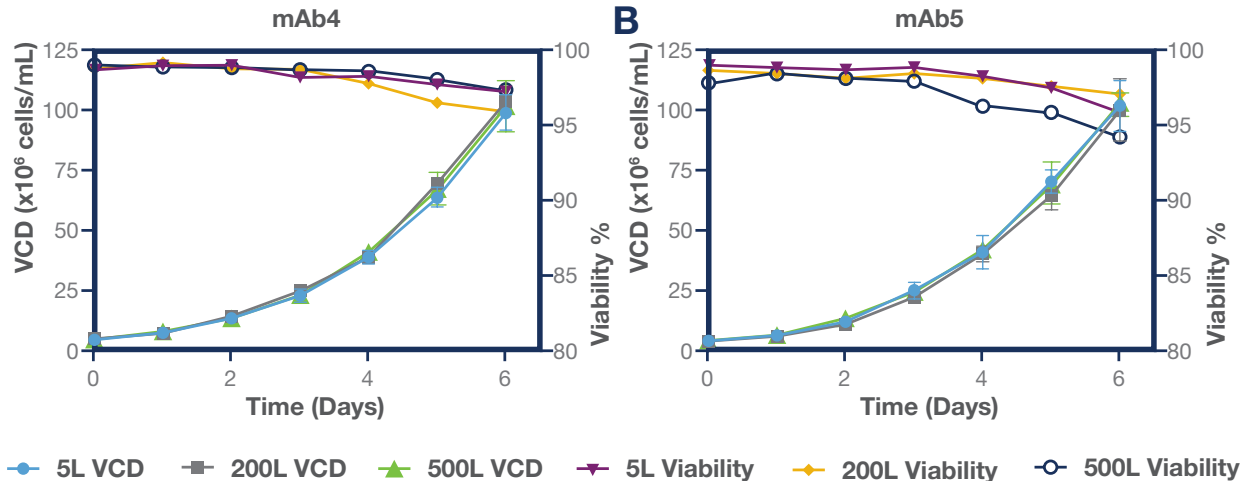


Figure 6: Scale up performance of N-1 perfusion bioreactors controlled by Incyte Arc (Hamilton) capacitance sensor. Shown are the viabilities of cell cultures at different bioreactor scales, measured using Hamilton's Incyte Arc cell viability sensor. VCD and viability are plotted for cell line 1 ((A); 5 L: n = 4, 200 L: n = 4, 500 L: n = 3) and cell line 2 ((B); 5 L: n = 5, 200 L: n = 1, 500 L: n = 3). Linearity of capacitance measurements (Incyte Arc) across different bioreactor scales. Figures adapted from[64].

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

4. Outlook

The outlook for this emerging industry is promising when we consider the value of learnings from related industries and research fields. For commercialization success, manufacturers should consider implementation of intelligent PAT such as intelligent sensors, which when connected to centralized control station can facilitate the automation within their production facilities, further improving the regulation, control, reliability, efficiency, reproducibility, and productivity of processes.

PAT in Production

During commercial production, the purpose of applying PAT is to ensure production processes adhere to the quality-by-design approach established during process design and optimization. Quality of the product is the focus, and in-line application of process analytical sensor technologies measuring critical process parameters (pH, DO, dissolved CO_2, temperature) and key process indicators (cell density) throughout production from primary locations at the bioreactor monitoring proliferation and differentiation, to secondary positions at perfusions systems providing media to the scaffolds during maturation into the final product. Additional secondary positions are media preparation tanks during water preparation, media preparation, media reclamation and water treatment. Throughout the white paper, the importance of measuring multiple critical process parameters due to the interplay between them has been exemplified through case studies. In this section, using published cell-culture case studies, we aim to inspire the cultivated meat industry as it advances towards more complex-structured meat products by demonstrating how the adoption of multiple in-line sensors measuring various critical parameters and process indicators could be used to ensure quality and yield of the structured meat products.

CASE STUDY

2D and 3D On-line Monitoring of Differentiation and Cell-line Expansion

This study demonstrated a method for monitoring the differentiation of hMSC cells in real-time following conductivity and capacitance of cells.

When monitoring in 2D in suspension culture (e.g., in the bioreactor), differences in conductivity and capacitance frequencies for cells differentiating into different lineages were observed, and these differences continue to change as differentiation and cellular specialization progress (Figure 7 A-D). Furthermore, when monitored at a constant frequency, clear differences were observed in both conductivity and capacitance measurements over time (Figure 7 E-F), indicating the value of such measurements for monitoring the differentiation of cells in real-time.

Monitoring in 3D where cells are supported by a static scaffold further supported 2D data as differences in conductivity was observed for the different cell types, and when measured from different angles (as indicated by the locations of the 4 electrodes (E1, E2, E3, E4) (Figure 7 G-I).

This study is an example of a proof-of concept for the ability to transition the same in-line PAT measuring principles from 2D dynamic suspension cell culture (in the bioreactor) during proliferation and differentiation stages to static 3D environments during maturation stages (adhered to scaffolds), further supported by this case

study. Furthermore, this study along with other studies[65] demonstrate the unique abilities of in-line conductance and capacitance sensors for monitoring cell differentiation at different stages during production processes, from proliferation to differentiation and maturation, without influence from media, microcarriers, dead cells or cell debris.

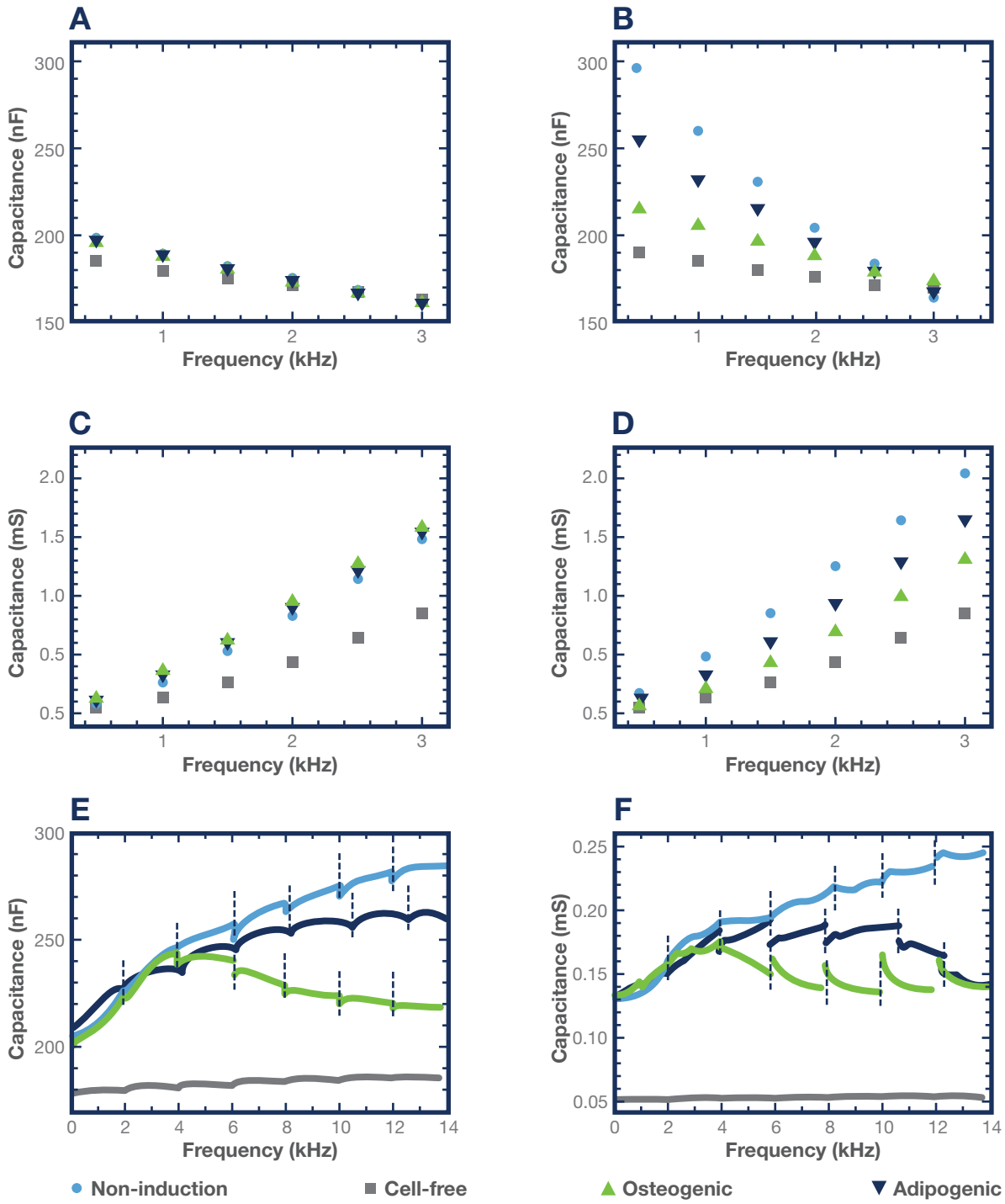


Figure 7: Real-time monitoring of hMSC capacitance (A-C) and conductance (D-F) in the 2D cell and capacitance (G-I) and conductance (J-L) in the 3D cell culture system at f = 0.5 kHz, measured using four electrodes, E1, E2, E3, and E4. Cell-free medium is the negative control in 2D and hydrogel in 3D. Figure adapted from[66].

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

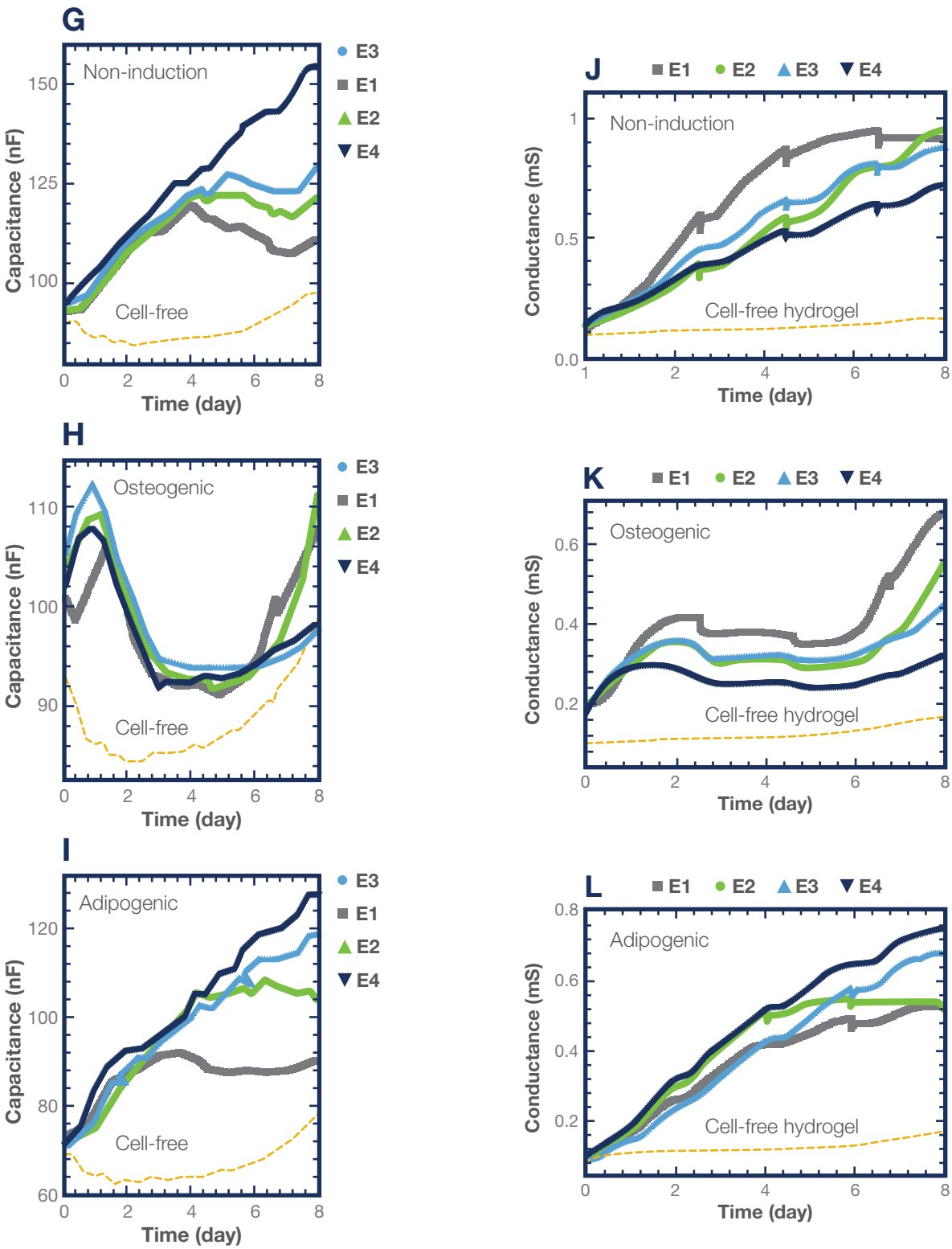


Figure 7: Real-time monitoring of hMSC capacitance (A-C) and conductance (D-F) in the 2D cell and capacitance (G-I) and conductance (J-L) in the 3D cell culture system at f = 0.5 kHz, measured using four electrodes, E1, E2, E3, and E4. Cell-free medium is the negative control in 2D and hydrogel in 3D. Figure adapted from[66].

Automation and Labor

Automation has the potential to improve the reliability, reproducibility, scalability, yield, quality, and efficiency (space, time, cost, resource) of processes. It is advised that automation is implemented into the manufacturing process for the beginning rather than retrospectively[67] to reduce costly process modifications and downtimes. One way to ensure consistency across each phase of production is to implement in-line PAT, as such measuring methods directly monitor parameters in the process for better understanding of their status in real-time. Furthermore, when using intelligent sensors for in-line measurements, this opens the possibility for automation with respect to parameter adjustment and progression of subsequent steps. Automation can only be implemented for in-line and to a lesser extent on-line measurements, as at-line and off-line measurements require manual handling and analysis of samples away from the bioreaction, therefore are unsuitable for automation.

Hamilton's Arc technology enables wireless connectivity between sensors and control systems. Arc-enabled sensors have Arc technology integrated into the sensor enabling localized data storage at the sensor and self-diagnostics, ensuring optimum sensor performance.

Implementation of in-line PAT and automation from the outset of a project has the benefit of round-the-clock monitoring of processes which can reduce delays and downtime during production often caused by manual intervention. Although the initial expense of specialist equipment built for in-line PAT and automation will be comparatively higher that more manual-labor intensive set-ups, the long-term benefits and assurance of process performance are outweighed in the long-term.

CASE STUDY

Evaluation and Scale-up of Continuous Bleed Recycling to Maximize Yield of Perfusion Cell Culture Processes

In this case study, a bleed recycling method of perfusion cell culture was used, whereby the bleed stream is separated into a concentrated waste stream and a separate debris-free recycle stream that is recirculated back to the bioreactor.

During a 42-day perfusion process at 36.5°C whereby a dissolved oxygen set-point of 50% was maintained using a VisiFerm DO sensor (Hamilton), a 3.5-fold bleed reduction and a 19% increase in average harvest rate was achieved with a capacitance-controlled (Incyte, Hamilton) bleed set-up. The bleed rate was controlled by in-line capacitance measurement (Incyte, Hamilton) which is unaffected by the presence of dead cells or cell debris, and automatically changed the waste and recycle stream flowrates. As a result, the recycle pump worked at a fraction of the process bleed pump. Furthermore, no operator intervention was necessary during the study (Figure 8). Together, this study is an example of how in-line measurements monitoring a critical process parameter enable automated adjustment of processes, at the fraction of personnel, resource, and utility expense of non-in-line-monitored processes.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

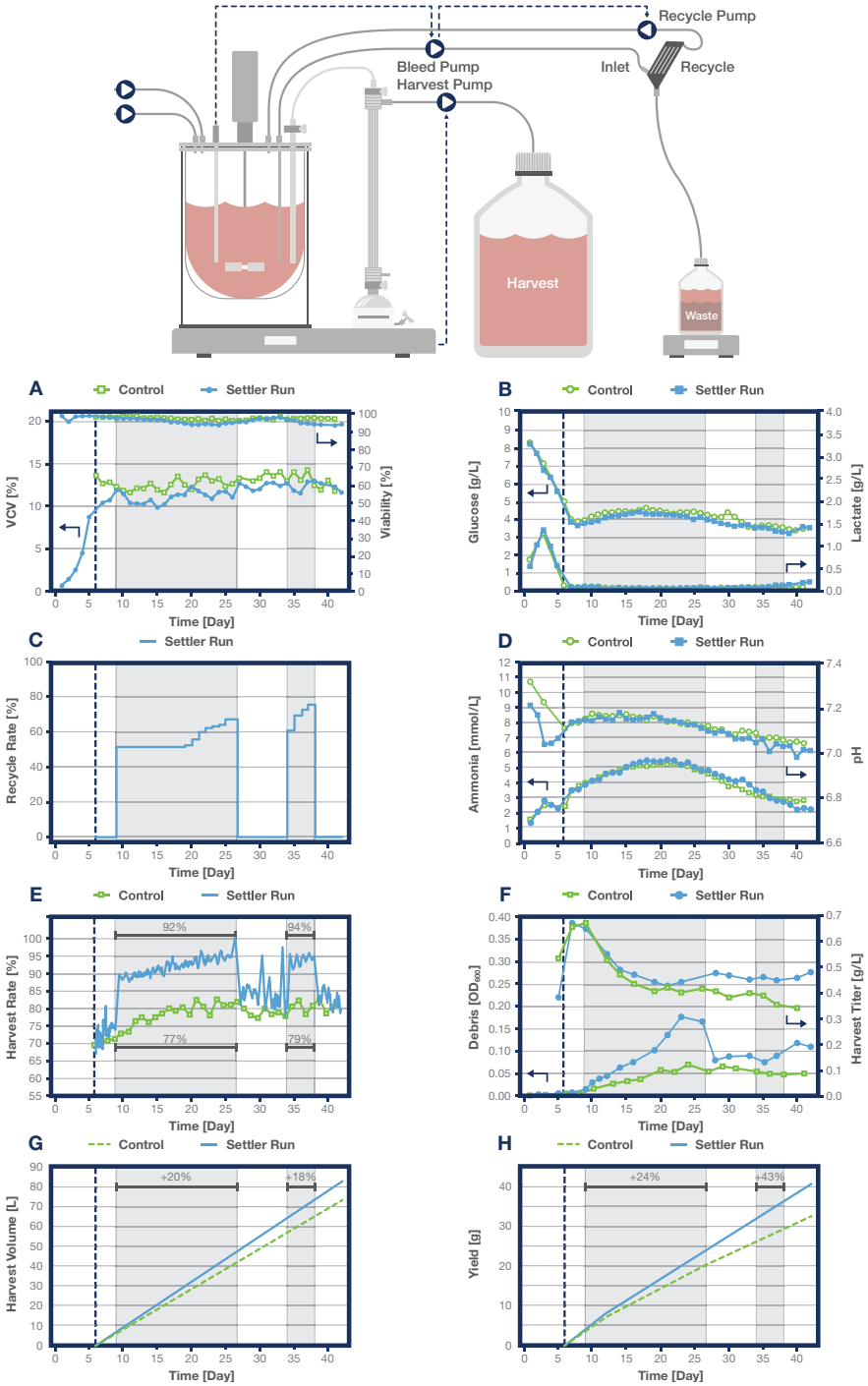


Figure 8: (Top) Schematic representation of the bleed recycling setup. (Bottom) Process data of lab-scale 2 L perfusion settler run compared with a standard control run without bleed recycling for mAb1. VCV and viability (A), glucose and lactate concentration (B), theoretical recycle rate setpoint (C), ammonia and pH profile (D), process harvest rate (E), harvest titer and debris trend (F), cumulative harvest volume (G) and cumulative process yield (H). Grey areas represent process phases were bleed recycling was performed, whereas bleed recycling was turned off during white process phases. pH was measured using an EasyFerm sensor (Hamilton), dissolved oxygen using a VisiFerm sensor (Hamilton), and cell viability/capacitance using an Incyte sensor (Hamilton). Figures from[68].

Implementation of in-line processes enables automated monitoring and response to parameter changes in processes. In such cases, this also brings with automation, a reduction in manual handling and therefore the associated personnel costs and potential for delays and human-error as demonstrated in the study by[68]. Furthermore, improved product yields are more achievable with automated set-ups due to the ability to continuously ensure conditions favorable for cell growth.

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

CASE STUDY

Novel, Customizable Perfusion Bioreactor System for Whole Heart Cultivation

This study successfully applied a central control algorithm for the automated, independent in-line control of critical process parameters including pH and pO_2 (Visiferm DO sensor, Hamilton) via variable gas mixing in the bioreactor (Figure 9), and they were able to dynamically control the bioreactor environment, e.g., make short-term changes to the pre-settings to induce specific conditions during cultivation of a whole heart.

Using a top-down approach, a decellularized heart was used as a scaffold onto which cells adhered to for growth. VisifermThis study provides a clear demonstration of the benefits of in-line monitoring of multiple process parameters for real-time feedback and automated responses in bioreactor processes, and the value of their application in the building of tissue structures on scaffolds.

In addition to pH and DO sensors, this study could have benefitted from the application of a capacitance probe measuring viable cell density such as Hamilton's Incyte Arc as an additional indicator of cell adherence and growth to the scaffold. Use of an in-line capacitance sensor would have also provided cell-growth data in real-time, could have been used to optimize the media conditions as an indicator for the favorability of conditions for cell growth (e.g., DO, DCO_2, nutrient availability, and to test the compatibility of different scaffold materials and/or media composition). Building on knowledge presented in other case studies, capacitance measurements are capable of distinguishing between different cell types; this presents and interesting case for the cultivated meat industry moving forward as more complex meat structures are developed.

Recent studies from the industry investigating the differentiation and longevity of adipose cells (rather that the usual focus of muscle tissues) under different conditions (media composition, presence or absence of microcarriers) used off-line microscopy methods for cell counting during lab-scale experiments [69] and presents an opportunity for analytical method development. In-line capacitance sensors could be implemented in the workflow to (1) measure cell viability in a longitudinal study and (2) follow differentiation of cells. Following this suggestion would reduce the need for additional manual sample manipulation (e.g., cell staining) for analysis and opens the potential for efficient scaling of processes from lab-scale as protocols are optimized, and this is all possible in the presence of microcarriers due to the unique properties of capacitance measurements. Utilizing the R&D potential of building a perfusion bioreactor the for the maturation of cell-scaffold interactions (presented in this case study) and the information supported in a previous case study regarding the application of capacitance measurements in 3D to monitor differentiation and proliferation of different cell types, this presents an inspiring combination of data for the advancement of cultivated meat protocols.

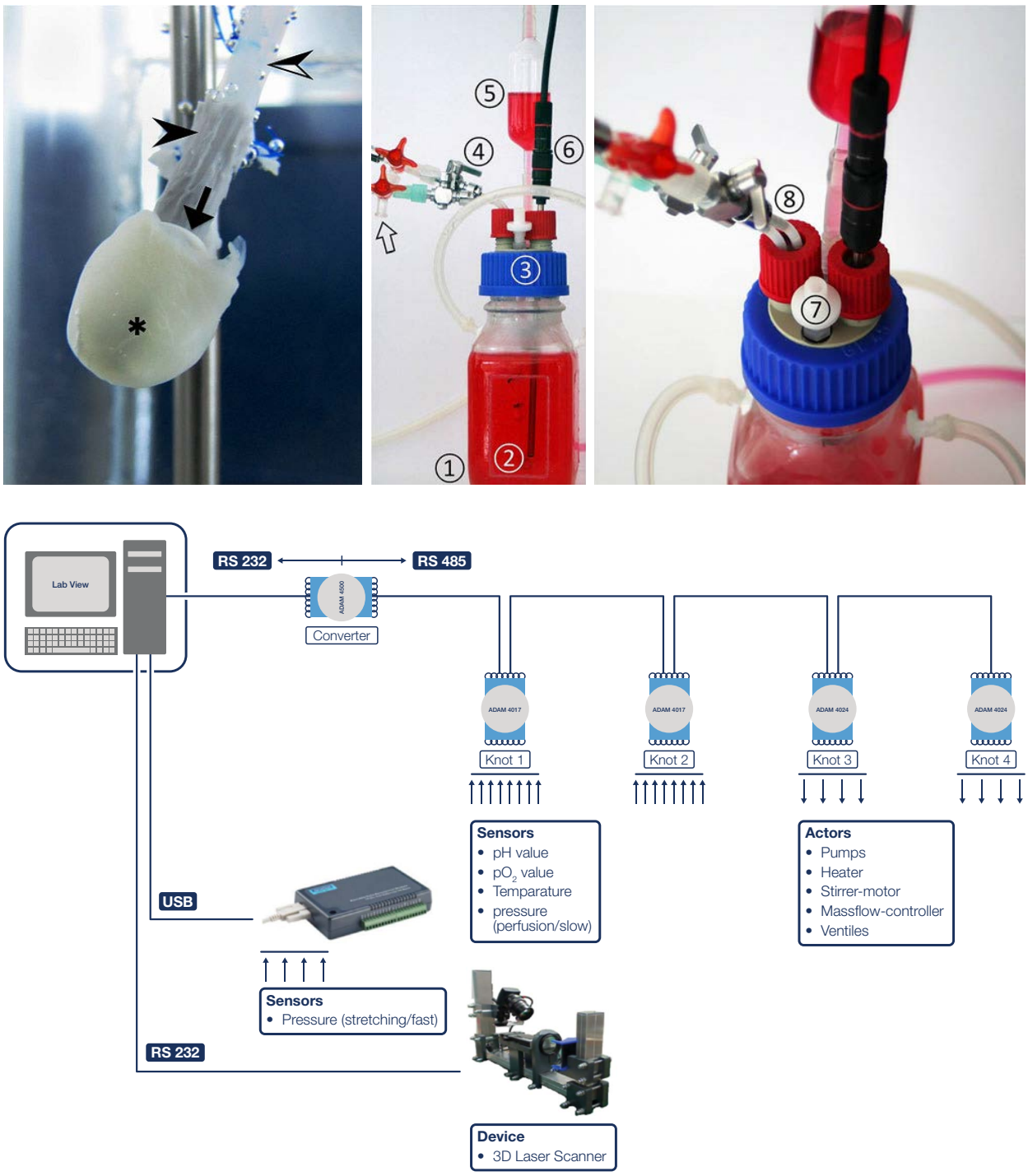


Figure 9: Set-up for the independent monitoring of process parameters in a customizable perfusion bioreactor for whole cell cultivation. (Left, Top) Close-up view of a decellularized rat heart mounted into the processing chamber. (Left, Bottom) Lateral view of the culture chamber, comprised of (1) 250 ml flask; (2) thin planar glass panel for optical accessibility and high precision optical analysis; (3) modified GL 45 2-Port lid; (4) cut-off cocks of the pressure pipes; (5) bubble trap; (6) pt-100 temperature sensor; (7) filter-equipped (0.2 µm) connection for pressure compensation; (8) pressure pipes for balloon expansion. (Right) Workflow for automation. Figures adapted from[70].

Check out our PAT sensors: Incyte Arc | CO_2NTROL | VisiFerm RS485 | EasyFerm Bio

5. Conclusion

The global market for cultivated meats is predicted to grow. At the time of writing, [50] companies were open globally, with 2 companies passing regulation in Singapore and the US and other companies not far behind [71]. This provides hope for a thriving future for this growing industry. As a new industry with many young companies offering several environmental, sustainable, and ethical benefits compared to traditional agricultural methods; there are still many opportunities for improvement. To improve the efficiency and profitability of cultivated meat production, companies should invest in developing and optimizing techniques that can be easily translated to large-scale production.

In-line Process Analytical Technologies (PAT) are essential for media and process developers as well as manufacturers to reliably and cost-efficiently increase production yields and quality. The critical process parameters necessary for optimization during cultivated meat production are the same as related cell culture applications, for which successfully offer a complete portfolio of sensor solutions. The usefulness of in-line sensors in accelerating innovation, efficiency, and productivity in related industries such as the cell culture and food industries are testament to their power when applied correctly.

For the cultivated meat sector, the focus is biomass production, therefore PAT providing insights into the number of cells at each stage of production is key. Total cell density (TCD) and viable cell density (VCD) are essential measurements that we at Hamilton offer, and have helped producers in the industry to implement into their production strategy. In-line process sensors continuously and accurately measure viable biomass throughout production, enabling immediate insights into the compatibility of conditions and areas requiring optimization in the bioprocess. Conditions can be monitored by measuring additional critical process parameters including dissolved CO_2, dissolved O_2 and pH. Dissolved CO_2 is an essential yet often overlooked parameter that is a contributing factor of low viable cell density when transitioning from lab-scale to commercial production scale. In-line PAT can be used to measure the efficiency of the entire process, therefore are instrumental for optimization and reducing the cost-economics of production: valuable for emerging industries such as the cultivated meat sector.

Technologies which are easily implement and transferable between scales and stages of production will be the most valuable for the cultivated meat industry as they can offer ease and continuity of control. For example, use of sensor solutions throughout all stages enables their connection to a single control system, enabling quick and easy comparisons of process performance throughout all stages of production. In-line methods offer low contamination risk and continuous, direct measurement of processes in real time process insights. They also open the potential for the integration of automatic process control systems, further reducing cost inputs for processes that have the potential for higher returns than more manual monitoring methods.

Costs associated with media production has been heavily covered in the literature, and for a valid reason. It is true that defining the specific raw materials and media components essential for cell growth to high-density in large volumes in artificial environments will be the turning point for media formulations, but it is important to also consider the specific cell type used. Creations of cell bank could help but is dependent on the cooperativity of independent companies and research groups, which may be difficult to coordinate in the real-world.

Ultimately, products of the cultivated meat industry are designed for consumption, therefore must be appealing, and accepted by consumers as without this the industry is without purpose. Defining and agreeing on the regulatory framework on a global scale will aid in the world-wide acceptance of cultivated meats as feasible future foods and will aid in customer acceptance. For customers, there are clear health benefits compared to traditional animal-reared meats including the advocacy of clean meat whereby no antibiotics are used during cell cultivation, and the improved animal welfare due no direct requirement for animal slaughter for meat.

The future of the cultivated meat industry is encouraging, as too are the possibilities from the wider cellular

5. Conclusion Cont.

agriculture industry. Production of other cellular products such as aquatic-animal products (fish and seafood), offal (including foie gras) and other less-common meats (such as duck, rabbit, horse, kangaroo and venison), in addition to eggs, chocolate, wood, leather, furs and silks are possible. Acellular products including milk and other dairy products, coffee, honey, food colorants, food flavorings, health-food ingredients and cosmetics ingredients are possibilities [72,73] for this growing industry.

The potential of products from this industry, improved ethics and animal welfare opportunities, and comparatively better sustainability of this emerging industry for delivering proteins sources to future generations is our motivation for helping to secure the future of this industry. For this, bioprocesses throughout production must be optimized. Harnessing our experiences and success in related cell culture applications, at Hamilton we suggest the implantation of in-line PAT as a tool for improving the efficiency of bioprocesses. We offer a comprehensive portfolio of bio-safe sensors suitable for cell culture and food applications beyond the essential capacitance sensors for measuring viable cell density which a have been proved as useful technologies to the cultivated meat industry, as demonstrated in our recent application note. We are excited for the development of this industry and look forward to future collaborations implementing our innovative technologies to this rising sector.

6. References

[1] Nations, U. World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100 | United Nations. (2017).

[2] Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M. & Tiwari, B. Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. Foods 6, 1–21 (2017).

[3] Bushnell, C., Specht, L. & Almy, J. 2022 Cultivated Meat State of the Industry Report. (2022).

[4] Post, M. J. et al. Scientific, sustainability and regulatory challenges of cultured meat. Nature Food 2020 1:7 1, 403–415 (2020).

[5] Allan, S. J., De Bank, P. A. & Ellis, M. J. Bioprocess Design Considerations for Cultured Meat Production With a Focus on the Expansion Bioreactor. Front Sustain Food Syst 3, (2019).

[6] Garrison, G. L., Biermacher, J. T. & Brorsen, B. W. How much will large-scale production of cell-cultured meat cost? J Agric Food Res 10, 100358 (2022).

[7] Specht, E. A., Welch, D. R., Rees Clayton, E. M. & Lagally, C. D. Opportunities for applying biomedical production and manufacturing methods to the development of the clean meat industry. Biochem Eng J 132, 161–168 (2018).

[8] FDA. Guidance for Industry PAT - A Framework for Innovative Pharmaceutical Development, manufacturing, and Quality Assurance. (2004).

[9] Workman, J., Lavine, B., Chrisman, R. & Koch, M. Process analytical chemistry. Anal Chem 83, 4557–4578 (2011).

[10] O'Donnell, C. P., Fagan, C. & Cullen, P. J. Process Analytical Technology for the Food Industry. (2014) doi:10.1007/978-1-4939-0311-5.

[11] van den Berg, F., Lyndgaard, C. B., Sørensen, K. M. & Engelsen, S. B. Process Analytical Technology in the food industry. Trends Food Sci Technol 31, 27–35 (2013).

[12] Fagan, C. C. et al. Novel Online Sensor Technology for Continuous Monitoring of Milk Coagulation and Whey Separation in Cheesemaking. J Agric Food Chem 55, 8836–8844 (2007).

[13] Panikuttira, B. et al. Investigation of an in-line prototype fluorescence and infrared backscatter sensor to monitor rennet-induced coagulation of skim milk at different protein concentrations. Int J Food Sci Technol 55, 175–182 (2020).

[14] Woodcock, T., Downey, G. & O'Donnell, C. P. Confirmation of Declared Provenance of European Extra Virgin Olive Oil Samples by NIR Spectroscopy. J Agric Food Chem 56, 11520–11525 (2008).

[15] Hennessy, S., Downey, G. & O donnell, C. P. Attempted Confirmation of the Provenance of Corsican PDO Honey Using FT-IR Spectroscopy and Multivariate Data Analysis. J Agric Food Chem 58, 9401–9406 (2010).

[16] Tiwari, B. K., O'Donnell, C. P., Brunton, N. P. & Cullen, P. J. Degradation kinetics of tomato juice quality parameters by ozonation. Int J Food Sci Technol 44, 1199–1205 (2009).

[17] Everard, C. D., Kim, M. S. & O'Donnell, C. P. Distinguishing Bovine Fecal Matter on Spinach Leaves Using Field Spectroscopy. Applied Sciences 2016, Vol. 6, Page 246 6, 246 (2016).

[18] Khanal, O. & Lenhoff, A. M. mAbs ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/kmab20 Developments and opportunities in continuous biopharmaceutical manufacturing. (2021) doi:10.1080/19420862.2021.1903664.

[19] Ündey, C., Ertunç, S., Mistretta, T. & Pathak, M. Applied Advanced Process Analytics in Biopharmaceutical Manufacturing: Challenges and Prospects in Real-time Monitoring and Control. IFAC Proceedings Volumes 42, 177–182 (2009).

[20] Shek, C. F., Betenbaugh, M., Rosenberg, J. N., Bentley, W. E. & Betenbaugh, M. J. Taking the pulse of bioprocesses: at-line and in-line monitoring of mammalian cell cultures $ This review comes from a themed issue on Analytical biotechnology. Curr Opin Biotechnol 71, 191–197 (2021).

[21] Hewitt, M. M., Trainor, N., Ostrout, N. & Abraham, E. Cell therapy manufacturing: process analytic technologies needed to achieve flexible, feedback-driven automation. (2021) doi:10.1016/j.cobme.2021.100358.

[22] Rathore, A. S. et al. Bioprocess Control: Current Progress and Future Perspectives. Life 2021, Vol. 11, Page 557 11, 557 (2021).

[23] Lee, K. Y., Loh, H. X. & Wan, A. C. A. Systems for Muscle Cell Differentiation: From Bioengineering to Future Food. Micromachines vol. 13 Preprint at https://doi.org/10.3390/mi13010071 (2022).

[24] Rao, L., Qian, Y., Khodabukus, A., Ribar, T. & Bursac, N. Engineering human pluripotent stem cells into a functional skeletal muscle tissue. Nature Communications 2018 9:1 9, 1–12 (2018).

[25] Chal, J. & Pourquié, O. Making muscle: skeletal myogenesis in vivo and in vitro. Development 144, 2104–2122 (2017).

[26] Chal, J. & Pourquié, O. Making muscle: skeletal myogenesis in vivo and in vitro. Development 144, 2104–2122 (2017).

[27] Specht, L. Cultivated meat: Cell culture medium costs | Analysis | GFI. The Good Food Institute https://gfi.org/resource/analyzing-cell-culture-medium-costs/ (2020).

[28] Ho, Y. Y. et al. Applications and analysis of hydrolysates in animal cell culture. Bioresources and Bioprocessing vol. 8 Preprint at https://doi.org/10.1186/s40643-021-00443-w (2021).

[29] O'Neill, E. N. et al. Spent media analysis suggests cultivated meat media will require species and cell type optimization. npj Science of Food 2022 6:1 6, 1–10 (2022).

[30] Andreassen, R. C., Pedersen, M. E., Kristoffersen, K. A. & Beate Rønning, S. Screening of by-products from the food industry as growth promoting agents in serum-free media for skeletal muscle cell culture. Food Funct 11, 2477–2488 (2020).

[31] Hou, Y., Yin, Y. & Wu, G. Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. Exp Biol Med 240, 997 (2015).

[32] O'Neill, E. N., Cosenza, Z. A., Baar, K. & Block, D. E. Considerations for the development of cost-effective cell culture media for cultivated meat production. Compr Rev Food Sci Food Saf 20, 686–709 (2021).

[33] Hubalek, S., Post, M. J. & Moutsatsou, P. Towards resource-efficient and cost-efficient cultured meat. Curr Opin Food Sci 47, 100885 (2022).

[34] Spivak-Kroizman, T. et al. Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. Cell 79, 1015–1024 (1994).

[35] Andrae, J. et al. Analysis of mice lacking the heparin-binding splice isoform of platelet-derived growth factor A. Mol Cell Biol 33, 4030–4040 (2013).

[36] Strassel, C., Gachet, C. & Lanza, F. On the Way to in vitro Platelet Production. Front Med (Lausanne) 5, 239 (2018).

[37] Baigger, A. et al. Characterization of induced pluripotent stem cell-derived megakaryocyte lysates for potential regenerative applications. J Cell Mol Med 22, 4545–4549 (2018).

[38] Liu, L. et al. Systematic evaluation of sericin protein as a substitute for fetal bovine serum in cell culture. Scientific Reports 2016 6:1 6, 1–10 (2016).

[39] Martínez-Mora, C. et al. Fibroin and sericin from Bombyx mori silk stimulate cell migration through upregulation and phosphorylation of c-Jun. PLoS One 7, (2012).

[40] Terada, S., Nishimura, T., Sasaki, M., Yamada, H. & Miki, M. Sericin, a protein derived from silkworms, accelerates the proliferation of several mammalian cell lines including a hybridoma. Cytotechnology 40, 3–12 (2002).

[41] Tsubouchi, K., Igarashi, Y., Takasu, Y. & Yamada, H. Sericin enhances attachment of cultured human skin fibroblasts. Biosci Biotechnol Biochem 69, 403–405 (2005).

[42] O'neill, E. N. et al. The effect of serum-free media on the metabolic yields and growth rates of C2C12 cells in the context of cultivated meat production. Future Foods 7, 100226 (2023).

[43] Messmer, T. et al. A serum-free media formulation for cultured meat production supports bovine satellite cell differentiation in the absence of serum starvation. Nature Food 2022 3:1 3, 74–85 (2022).

[44] Okamoto, Y., Haraguchi, Y., Sawamura, N., Asahi, T. & Shimizu, T. Mammalian cell cultivation using nutrients extracted from microalgae. Biotechnol Prog 36, e2941 (2020).

[45] David, S. et al. Co-culture approaches for cultivated meat production. Nature Reviews Bioengineering 2023 1–15 (2023) doi:10.1038/s44222-023-00077-x.

[46] Galbraith, S. C., Bhatia, H., Liu, H. & Yoon, S. Media formulation optimization: current and future opportunities. Current Opinion in Chemical Engineering vol. 22 42–47 Preprint at https://doi.org/10.1016/j.coche.2018.08.004 (2018).

[47] Ryder, A. G. Cell culture media analysis using rapid spectroscopic methods. Curr Opin Chem Eng 22, 11–17 (2018).

[48] Johnson, C. H., Ivanisevic, J. & Siuzdak, G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 17, 451–459 (2016).

[49] Shek, C. F. & Betenbaugh, M. Taking the pulse of bioprocesses: at-line and in-line monitoring of mammalian cell cultures. Current Opinion in Biotechnology 191–197 https://sci-hub.se/10.1016/j.copbio.2021.08.007 (2021).

[50] Pawitan, J. A. Prospect of stem cell conditioned medium in regenerative medicine. Biomed Res Int 2014, (2014).

[51] Desai, N., Rambhia, P. & Gishto, A. Human embryonic stem cell cultivation: historical perspective and evolution of xeno-free culture systems. Reprod Biol Endocrinol 13, (2015).

[52] Thomson, J. A. et al. Embryonic Stem Cell Lines Derived from Human Blastocysts. Science (1979) 282, 1145–1147 (1998).

[53] Torizal, F. G. et al. A miniature dialysis-culture device allows high-density human-induced pluripotent stem cells expansion from growth factor accumulation. Communications Biology 2021 4:1 4, 1–13 (2021).

[54] Kämpfer, A. A. M. et al. Development of an in vitro co-culture model to mimic the human intestine in healthy and diseased state. (2017) doi:10.1016/j.tiv.2017.08.011.

[55] Lynch, C. D. & O'Connell, D. J. Conversion of mammalian cell culture media waste to microbial fermentation feed efficiently supports production of recombinant protein by Escherichia coli. PLoS One 17, e0266921 (2022).

[56] Nath, S. C., Nagamori, E., Horie, M. & Kino-oka, M. Culture medium refinement by dialysis for the expansion of human induced pluripotent stem cells in suspension culture. Bioprocess Biosyst Eng 40, 123–131 (2017).

[57] Kim, S., Beier, A., Schreyer, H. B. & Bakshi, B. R. Environmental Life Cycle Assessment of a Novel Cultivated Meat Burger Patty in the United States. Sustainability (Switzerland) 14, (2022).

[58] Meyer, H.-P., Minas, W. & Schmidhalter, D. Industrial-Scale Fermentation. Industrial Biotechnology 1–53 (2016) doi:10.1002/9783527807833.CH1.

[59] Barzee, T. J. et al. Cell-cultivated food production and processing: A review. Food Bioengineering 1, 4–25 (2022).

[60] Samandari, M. et al. Repurposing biomedical muscle tissue engineering for cellular agriculture: challenges and opportunities. Trends Biotechnol 41, 887–906 (2023).

[61] Brunner, M., Fricke, J., Kroll, P. & Herwig, C. Investigation of the interactions of critical scale-up parameters (pH, pO 2 and pCO 2) on CHO batch performance and critical quality attributes. Bioprocess Biosyst Eng 40, (2017).

[62] Paul, K., Hartmann, T., Posch, C., Behrens, D. & Herwig, C. Investigation of cell line specific responses to pH inhomogeneity and consequences for process design. Eng Life Sci 20, 412–421 (2020).

[63] Brunner, M., Doppler, P., Klein, T., Herwig, C. & Fricke, J. Elevated pCO2 affects the lactate metabolic shift in CHO cell culture processes. Eng Life Sci 18, 204–214 (2018).

[64] Rittershaus, E. S. C. et al. N-1 Perfusion Platform Development Using a Capacitance Probe for Biomanufacturing. Bioengineering 9, (2022).

[65] Isidro, I. A. et al. Online monitoring of hiPSC expansion and hepatic differentiation process in a 3D culture system by dielectric spectroscopy. Biotechnol Bioeng 118, 3610–3617 (2021).

[66] Song, J. H., Lee, S.-M. & Yoo, K.-H. Label-free and real-time monitoring of human mesenchymal stem cell differentiation in 2D and 3D cell culture systems using impedance cell sensors †. (2018) doi:10.1039/c8ra05273e.

[67] Ball, O., Robinson, S., Bure, K., Brindley, D. A. & Mccall, D. Bioprocessing automation in cell therapy manufacturing: Outcomes of special interest group automation workshop. Cytotherapy 20, 592–599 (2018).

[68] Romann, P. et al. Maximizing yield of perfusion cell culture processes: Evaluation and scale-up of continuous bleed recycling. Biochem Eng J 193, 108873 (2023).

[69] Hubalek, S., Melke, J., Pawlica, P., Post, M. J. & Moutsatsou, P. Non-ammoniagenic proliferation and differentiation media for cultivated adipose tissue. Front Bioeng Biotechnol 11, 1202165 (2023).

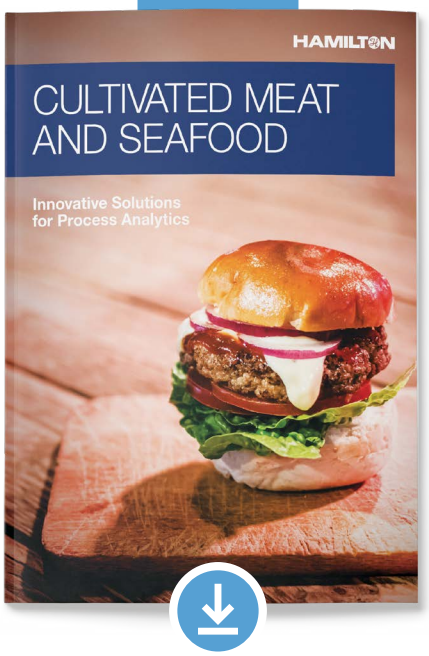
[70] Hülsmann, J. et al. A novel customizable modular bioreactor system for whole-heart cultivation under controlled 3D biomechanical stimulation. Journal of Artificial Organs volume 294–203 (2013) doi:10.1007/s10047-013-0705-5.

[71] Jones, N. Lab-grown meat: the science of turning cells into steaks and nuggets. Nature 619, 22–24 (2023).

[72] Gubser, G., Vollenweider, S., Eibl, D. & Eibl, R. Food ingredients and food made with plant cell and tissue cultures: State-of-the art and future trends. Engineering in Life Sciences vol. 21 87–98 Preprint at https://doi.org/10.1002/elsc.202000077 (2021).

[73] Eibl, R. et al. Plant cell culture technology in the cosmetics and food industries: current state and future trends. Applied Microbiology and Biotechnology vol. 102 8661–8675 Preprint at https://doi.org/10.1007/s00253-018-9279-8 (2018).

Related Hamilton Articles



HAMILTON

Web: www.hamiltoncompany.com
USA: 800-648-5950
Europe: +41-58-610-10-10

Hamilton Americas & Pacific Rim
Hamilton Company Inc.
4970 Energy Way
Reno, Nevada 89502 USA
Tel: +1-775-858-3000
Fax: +1-775-856-7259
sales@hamiltoncompany.com

Hamilton Europe, Asia & Africa
Hamilton Bonaduz A.G.
Via Crusch 8
CH-7402 Bonaduz, Switzerland
Tel: +41-58-610-10-10
contact.pa.ch@hamilton.ch

To find a representative in your area, please visit: www.hamiltoncompany.com/contacts