What are the critical risks in the cultured meat production process, and how can Safe-by-Design principles contribute to mitigating these risks to meet EU safety, ethical, and regulatory standards?

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Table of Contents

1. ABSTRACT 3

2. INTRODUCTION 4

3. CULTURED MEAT AS A NOVEL FOOD IN THE EU 5

4. EU REGULATIONS 6

4.1 Timeline for Novel Food Market Approval 6
4.2 Headlines EU regulation 8
4.3 Bottlenecks in EU Novel Foods Approval for Cultivated Meat 9

5. SAFE BY DESIGN IN THE CULTIVATED MEAT PRODUCTION PROCESS 10

6. IDENTIFYING RISKS AND MITIGATION STRATEGIES IN THE CM PRODUCTION PROCESS 12

6.1. Cell sourcing 14
6.2. Cell culture development 19
6.3. Growth media: Risks, ethical and safety concerns 24
6.4. Cell proliferation 27
6.5. Bioreactors' challenges in scale-up 29
6.6. Cell differentiation 31
6.7. Cell harvesting and separation 34

7. INTERVIEW 35

8. CONCLUSION 38

9. REFERENCE LIST 41

1. Abstract

The meat industry is a major contributor to greenhouse gas emissions, resource depletion, and ethical concerns, which highlights the need for sustainable and innovative alternatives to ensure food security. Cultured meat, produced by in vitro cultivation of animal cells, presents a promising alternative to conventional meat production. Cultivated meat could offer potential reductions in land use, water consumption, and greenhouse gas emissions. However, its development and market acceptance face numerous challenges, including microbial contamination, allergenicity, scalability issues, and the strict European Union (EU) regulatory framework for novel foods.

This research addresses the critical risks associated with the lab-grown meat production process and explores how Safe-by-Design principles can mitigate these risks to meet EU safety, ethical, and regulatory standards. SbD focuses on integrating safety measures within the whole production process, including cell sourcing, culture development, proliferation, differentiation, and bioreactor design. This study identifies key risks, such as contamination, genetic instability, and ethical concerns, and proposes solutions like serum-free media, optimised bioreactor systems, and improved cell differentiation techniques. Furthermore, it discusses the bottlenecks of the EU's regulatory approval process and evaluates how SbD can help streamline compliance.

By applying SbD principles, this research offers practical recommendations for improving the safety, efficiency, and public acceptance (by addressing ethical concerns) of cultivated meat. These insights aim to support researchers, policymakers, and industry leaders in fostering a sustainable and ethical transition within the EU to cultivated meat.

2. Introduction

The global food industry is a major contributor to greenhouse gas (GHG) emissions and contributes to the current issue of climate change. Livestock production, deforestation, and food transportation together account for 11% of the annual GHG emissions in the European Union (European Environment Agency, 2023). Agriculture generates 94% of the EU's ammonia (NH_3) emissions (European Environment Agency, 2024). Also, the global population is expected to reach 9.7 billion by 2050 (United Nations, 2022). Therefore, the demand for food will also rise, which will lead to an increase in agricultural activity, also including livestock farming. This industry is resource intensive and requires significant amounts of land and water whilst contributing to deforestation and GHG emissions. There is an urgent need to address climate change, resource scarcity, and ethical concerns in meat production. Therefore, finding sustainable alternatives is very important for ensuring global food security and environmental sustainability.

Cultured meat, also called lab-grown, cell-based, or cultivated meat, could offer a promising solution to these environmental challenges of the current meat industry (Kirsch et al., 2023) and could also offer a solution to the ethical concerns of the industry. This cultivated meat technology involves cultivating animal cells in a controlled environment to produce meat without the need for raising and slaughtering animals. It has the potential to significantly reduce the environmental footprint of meat production. Studies suggest reductions of up to 99% in land use and 96% in water use compared to traditional methods (Tuomisto & Teixeira de Mattos, 2011). Moreover, this innovative method has the potential to lower greenhouse gas emissions and also lower the need for antibiotics and hormones in meat production, which would mitigate health risks associated with antibiotic resistance (Lanzoni et al., 2024). However, the production process does not come without its risks, including bacterial contamination, potential allergenicity, and scalability challenges. These risks would require careful management to ensure safety and consumer acceptance.

The EU presents additional challenges because of its stringent regulatory framework for novel foods, like cultured meat (Lanzoni et al., 2024) and is therefore regulated under the Novel Food Regulation (Regulation (EU) 2015/2283) (European Parliament and Council of the European Union, 2018). This regulation states that any ‘novel foods' not consumed in the EU before May 15, 1997, must undergo a rigorous safety assessment and obtain authorisation before getting market approval. These assessments are carried out by the European Food Safety Authority (EFSA), they evaluate the composition of the product, nutritional value, potential toxicity, and allergenicity. Only after receiving a positive evaluation from the EFSA a cultured meat product can be authorised for market entry in the EU. The final approval will also depend on political and ethical considerations, setting scientific evaluations aside (Lanzoni et al., 2024). This current situation calls for a structured approach to identifying and managing risks in alignment with regulatory requirements.

To address these challenges, a Safe-by-Design approach could be a step in the right direction for gaining market approval of CM. SbD entails a proactive and iterative approach to embedding safety, in this case, into the cultured meat production. While the EU regulations emphasise a proactive approach to food safety, traditional methods like final-product testing

and standard risk assessments may fall short in addressing the unique challenges and novel aspects of CM production. Therefore, adopting an SbD approach could better comply with these regulatory expectations by embedding safety considerations early in the design process. Through iterative design loops, SbD enhances risk assessment and management by ensuring that safety is an integral design goal from the start (Robaey, 2018). The use of an SbD approach is novel in the CM production process, and therefore, this concept offers a potential solution to the regulatory challenges and stringent food and safety standards presented by the EU by allowing the early identification and mitigation of risks such as bacterial contamination, allergens, and other hazards. Additionally, animal welfare is a critical ethical concern; SbD could integrate ethical objectives. This dual focus on safety and ethics sets an SbD approach as a potential solution to the regulatory and societal challenges faced by CM.

Cultured meat holds the potential to become a sustainable and ethical alternative to conventional meat production, but its successful market introduction depends on addressing critical safety, regulatory, and ethical challenges. This study aims to explore the critical risks associated with cultured meat production and evaluate how SbD principles can mitigate these risks to meet EU safety, ethical, and regulatory standards. By conducting a comprehensive risk identification of the production process and validating findings through expert interviews, this research seeks to identify actionable insights to support policymakers, producers, and regulators**.** Ultimately, this study aims to address environmental, ethical, and safety challenges to facilitate the safe and sustainable market introduction of cultured meat in Europe.

3. Cultured meat as a novel food in the EU

Cultured meat is produced without the need for animal slaughter; instead, it is grown in vitro. The production process consists of four main stages, starting with cell sourcing. In this step, a biopsy is taken from a selected animal to obtain the cells needed for the cultivation. Once sourced, the cells undergo proliferation and differentiation, during which desirable cells are isolated and placed into bioreactors containing the necessary stimuli to ensure growth, viability, and differentiation. The growth medium not only supplies essential nutrients but must also include critical components such as proteins, peptides, growth factors, and hormones (Lanzoni et al., 2024).

Since scaffolding is not yet being used in the commercial production of CM, this research will focus instead on the production of unstructured meat products, such as ground meat for hamburgers or chicken nuggets (Swartz, 2024). Once the cultured meat has been fully developed, it must meet strict safety standards to ensure it is suitable for human consumption. Risks can emerge throughout the production process and extend into distribution and storage. For example, microbial contamination during cell culture, unintended chemical residues from the growth medium, or breakdowns in cold-chain storage could compromise the end product. Therefore, identifying and finding ways to mitigate risks at every stage of the production process is essential to ensure consumer safety.

In the EU, the regulation of cultured meat falls under the framework for novel foods. This regulation defines novel foods as foods not significantly consumed in the EU before May 15,

1997, or foods produced using methods developed after this date. Cultivated meat, being a product of cell culture technology, meets these criteria. That is why CM must undergo rigorous safety assessments conducted by the EFSA to ensure compliance with EU food safety standards. Only after successfully passing these evaluations can the product be included in the "Union List" of authorised novel foods, which permits its marketing across all EU member states. While the regulatory framework ensures a comprehensive safety evaluation, it also presents a challenge. EFSA's assessment process is highly rigorous and can, therefore, be really time-consuming and resource-intensive for producers of CM, potentially delaying the introduction of cultured meat to the market.

Cultured meat has the potential to transform the global food system by offering a sustainable and ethical alternative to conventional animal farming. However, its classification as a novel food in the EU underscores the complexities of its production, safety, and market introduction. Balancing regulatory compliance, consumer acceptance, and innovation will be essential to achieving the consumption of cultured meat within the EU food market.

4. Eu regulations

The key objective of this regulatory framework, created to govern novel foods by the EU, is to foster innovation in the food sector while still ensuring consumer safety. The regulation mandates that cultivated meat must undergo a rigorous safety assessment, it emphasises consumer protection and ensures that the novel foods will be labelled accordingly to distinguish them from non-novel foods. While comprehensive, the process is often criticised for its complexity and the length of time required for approval, which counteracts the steps towards more sustainable agriculture (The Parliament Magazine, n.d.).

4.1 Timeline for Novel Food Market Approval

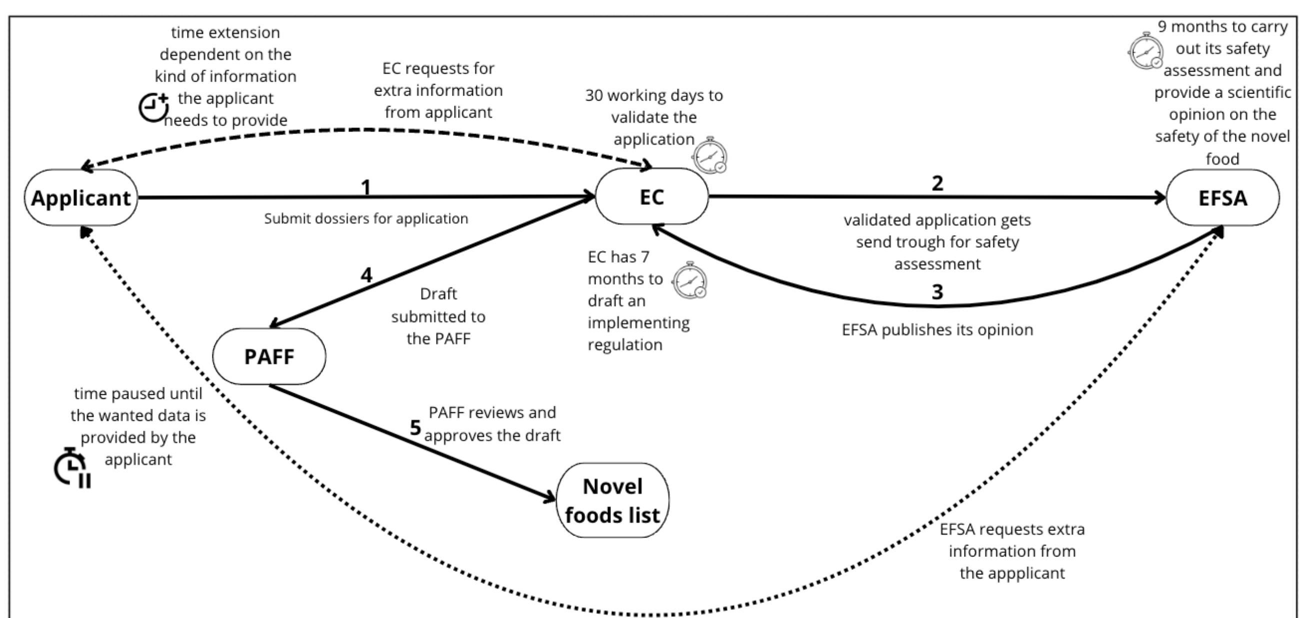


Figure 1: An overview of the EU regulation for the application process of novel foods for inclusion on the novel foods list (Regulation (EU) 2015/2283).

The process for obtaining authorisation for a novel food in the EU begins with the submission of a detailed dossier by the applicant to the European Commission (EC). The EC then forwards the application to EFSA for evaluation. This dossier must include a comprehensive description of the whole manufacturing process; of the food, its production methods, and a detailed risk assessment. In the case of cultured meat, the production company must provide information on the source of the cells (e.g., muscle stem cells from chicken), the production process used for cell culture growth, the bioreactor systems and setups employed, and any scaffolds, growth factors, or biological substances involved (Regulation (EU) 2017/2469). Also, the applicant must demonstrate that the production process does not introduce harmful microbes or pathogens into the food, particularly given that cultured meat is not subjected to the same environmental conditions as conventionally slaughtered animals. This includes information on contamination prevention methods and sterilisation procedures within the bioreactor systems (EFSA NDA Panel, 2024).

Essential in the dossier is the inclusion of the toxicology data, this section provides studies and analyses that demonstrate the novel food does not contain any harmful substances or compounds. In the case of cultured meat, this includes assessments of the safety of the growth media and the cell line stability (if cell lines mutate during the proliferation phase, this could potentially result in the production of harmful proteins or compounds that might have toxic effects when consumed) (Identification of Hazards in Meat Products Manufactured From Cultured Animal Cells: Hazards, 2023). The dossier must also include the applicant's data on any processing aids or chemicals that could be introduced during manufacturing and remain in the final product, such as antibiotics and hormones (Lanzoni et al., 2024).

Another significant component of the dossier is the nutritional assessment, the nutritional profile of the cellular meat needs to be identified, this involves an analysis of protein content, fats, vitamins, and minerals. The EFSA requires this information to determine whether the novel food meets the same nutritional needs as traditional meat products. If there are significant differences, the dossier must explain these variations and provide scientific evidence that the novel food is still safe and nutritious for consumers. The dossier must also include an allergenicity assessment. In the case of cultured meat, using growth factors, hormones, or other biologically active substances in the cell culture medium could trigger allergic reactions. Therefore, the applicant must provide data from example clinical trials or laboratory studies that assess the likelihood of allergic responses in sensitive populations (EFSA NDA Panel, 2024).

EFSA's evaluation also considers the potential for long-term effects, meaning whether the consumption of the novel food could, over time, lead to any long-term health impacts that might not be immediately apparent. This section of the dossier requires the applicant to provide any available data on long-term consumption studies or projections, although these are often challenging to produce for entirely new foods (EFSA, 2021).

Once the dossier is submitted by the applicant, the EC must verify whether the application meets the requirements outlined in the regulation (Figure 1, Step 1). The Commission can

consult the EFSA during this phase, and the validity of the application must be determined within 30 working days. The validation time can be extended because the EC has the right to request additional information from the applicant if there are uncertainties or gaps, in which case the validation clock will be paused. The EC and the applicant need to agree on the timeframe in which the applicant will provide the EC with the additional information. Thus, the 30-day period is not always a fixed duration and can be prolonged until the required information is received and reviewed.

After the validation period and if accepted, the EC will send the application forward to the EFSA, which will start the risk assessment phase, which has a nine-month timeframe (Figure 1, Step 2). The EFSA will need to carry out its safety assessment and provide a scientific opinion on the safety of the novel food. These nine months could also be extended if the EFSA requires more information from the applicant during the review. If additional information is requested, the EFSA's timeline can be paused until the applicant submits the required data. The process until the EFSA completes its safety assessment and delivers an opinion on the novel food, with the publication of the EFSA's scientific opinion, has a total minimum timeframe of 9 months (Regulation (EU) 2015/2283).

After the EFSA publishes its opinion on a novel food application, the EC has seven months to draft an Implementing Regulation (Figure 1, Step 3). This draft is then submitted to the Standing Committee on Plants, Animals, Food, and Feed (PAFF Committee) (Figure 1, Step 4). The PAFF Committee must review and approve the draft before the novel food can be added to the Union list of authorised novel foods (Figure 1, Step 5). Without extensions, the total minimum timeframe for this process is approximately one and a half years, but delays are common, especially when additional data is requested (Regulation (EU) 2015/2283). The framework is essential to guarantee food safety on the up-and-coming food source of cultivated meat. The regulation is, therefore, quite tedious in some aspects and can be depicted as overly complex and slow.

4.2 Headlines EU regulation

In the regulation structure of the EU, there are a few key points that need to be addressed. To obtain approval for a product within the EU, specific requirements must be met as outlined by the regulations. These requirements apply to various steps in the process and are briefly summarised in Table 1. Detailed explanations of these steps are beyond the scope of this research but can be found in the article "Guidance on the scientific requirements for an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283"((EFSA Panel on Nutrition, Novel Foods and Food Allergens [NDA] et al., 2024).

Table 1: Overview of production steps, regulatory requirements, and strategies for compliance in novel food development

Step in Process	Requirement	How to Meet Requirement
Cell Sourcing	Identity of source organism, genetic and phenotypic identity of cells, biopsy location, and absence of zoonotic agents (e.g., viruses, prions).	Perform genetic and phenotypic analysis of source cells, document biopsy source, and conduct pathogen screening (e.g., for prions and viruses).
Cell Culturing	Detailed description of processes, including all input materials, safety	Develop a detailed process flowchart, identify critical control points, and

	hazards, HACCP compliance (principles in line with Regulation (EC) No 852/2004 on the hygiene of foodstuff), and operational controls.	implement HACCP (hazard analysis critical control point) and ISO (International Organisation for Standardisation) protocols.
Raw Material Preparation	Specification, regulatory status, and quality of all input materials, including processing aids, plastics, and fermentation materials.	Ensure compliance with EU material safety regulations, test for contaminants, and validate material quality through certification.
Product Storage	Evaluate chemical, microbiological stability, and degradation risks under storage conditions; propose shelf life.	Conduct stability tests under various storage conditions, monitor degradation markers, and establish shelf-life parameters.
Compositional Analysis	Quantitative and qualitative analysis of the food's components, including contaminants, allergens, and nutritional profile.	Analyse at least five batches for contaminants, allergens, and nutritional consistency using validated methods.
Final Product Specifications	Chemical, nutritional, and microbiological parameters with acceptable ranges/limits, validated through batch testing.	Define specification limits and validate them through batch-to-batch analysis, ensuring reproducibility.
Production Safety Management	Implementation of HACCP and ISO principles for food safety during production; identification of critical control points.	Integrate HACCP plans and ISO standards into production, focusing on critical control points and verification procedures.
Processing Aids	Information on processing aids like enzymes; experimental verification of their removal or safety evaluation if present.	Provide data on the removal or inactivation of enzymes through experimental tests in multiple production batches.
Product Use and Population	Description of intended use and population (e.g., general, adults, or specific groups); intake estimation.	Define usage scenarios, estimate intake per population group, and document target populations with safety considerations.
Exposure Assessment	Chronic and acute intake estimates based on proposed uses; exposure levels compared to health-based guidance values (HBGV).	Use FAIM or DietEx tools to calculate intake estimates, and compare exposure data with HBGVs to assess safety thresholds.
Safety Precautions	Guidance on preparation, populations to avoid consumption, and safety-based usage restrictions.	Develop preparation guidelines, specify restricted populations, and include safety-based instructions with the product.

4.3 Bottlenecks in EU Novel Foods Approval for Cultivated Meat

The EU has different regulations than other countries, such as Singapore and the United States, the EU's regulatory process is slower and more complex. In Singapore, the approval pathway is streamlined under the Singapore Food Agency (SFA), which conducts a single agency review that allows cultivated meat products to move quickly from assessment to

approval. Similarly, in the United States, a dual approach by the FDA and USDA divides responsibilities, with the FDA overseeing safety assessments and the USDA managing facility inspections and labelling, which together expedite decisions on cultivated meat products (Good Food Institute, 2023). These faster timelines make Singapore and the U.S. more attractive destinations for companies focused on bringing cultivated meat to market efficiently, leaving the EU at a competitive disadvantage (foodnavigator.com, 2023).

The EU's lengthy and complex regulatory process also has economic consequences in terms of funding and investment. Investors often hesitate to fund cultivated meat ventures in the EU because the prolonged timeline increases financial risk. Startups, which frequently rely on external capital for research and regulatory compliance, struggle with sustaining operations without revenue over a long period. Investors in other regions, where regulatory pathways are shorter and more predictable, are more likely to see quicker returns, creating an investment shift away from the EU and towards markets with faster approval processes (Nowshin, 2023). The EU's regulatory framework not only affects funding but also impacts sustainability goals and the EU's role in global food innovation. Cultivated meat is seen as a potential solution to reduce the environmental impact of traditional meat production. Still, the slow approval process delays its availability, hindering progress toward climate goals. Also, the EU's cautious approach discourages innovation within Europe, as companies are applying in regions with more supportive regulatory environments.

Another regulatory bottleneck in the EU is the ambiguity around products involving genetic engineering. Companies face additional regulatory hurdles under the EU's GMO regulations if genetic modification is used, such as in enhancing cell lines for efficiency. This requires separate safety evaluations and can add years to the approval timeline, further complicating market entry. The EU's cautious stance on GMOs, while intended to ensure public safety, adds layers of complexity and cost that may discourage companies from pursuing certain technological innovations within the EU market (Gfiapacadmin, 2024).

The cumulative effect of these bottlenecks highlights the need for potential reforms in the EU's regulatory framework. Streamlining EFSA's assessment process, adopting risk-based approval pathways for low-risk novel foods, and introducing fast-track options for sustainability-focused products like cultured meat could improve the system's efficiency. Such changes would help the EU retain its competitiveness in the global food innovation sector while maintaining high safety standards.

5. Safe by design in the cultivated meat production process

As stated in the introduction of the research, SbD is a proactive approach to risk management and product development. It emphasises integrating safety considerations throughout the entire lifecycle of a product, which is particularly critical in the context of cultivated meat. Ensuring consumer safety and achieving regulatory compliance are essential challenges in the production of cultured meat. Rather than retrofitting safety solutions after risks emerge, the SbD method seeks to anticipate risks from the start of the process at every

production step, thereby simplifying regulatory processes in the European Union and ensuring a safer end product.

In the cultivated meat context, there are three key aspects of implementing SbD: formulating SbD strategies, establishing processes to support these strategies, and ensuring that responsibilities are clearly distributed among stakeholders. The formulation of SbD strategies involves developing specific approaches to embed safety measures into product design from the beginning, to minimise risks at later stages. This proactive mindset encourages the early and active consideration of safety concerns during the design phase, reducing the likelihood of harmful effects during production or after the product reaches consumers (Robay, 2018).

SbD could not only address technical safety concerns but can also act as a bridge to consumer trust by embedding transparency and ethical considerations into the production process. By proactively identifying risks and engaging with stakeholders, SbD ensures that cultivated meat products are safer and more acceptable to the public, also clear labelling should enhance cinsumer trust on the long run. The integration of societal values such as sustainability, transparency, and cruelty-free production into SbD principles is crucial for gaining public confidence.

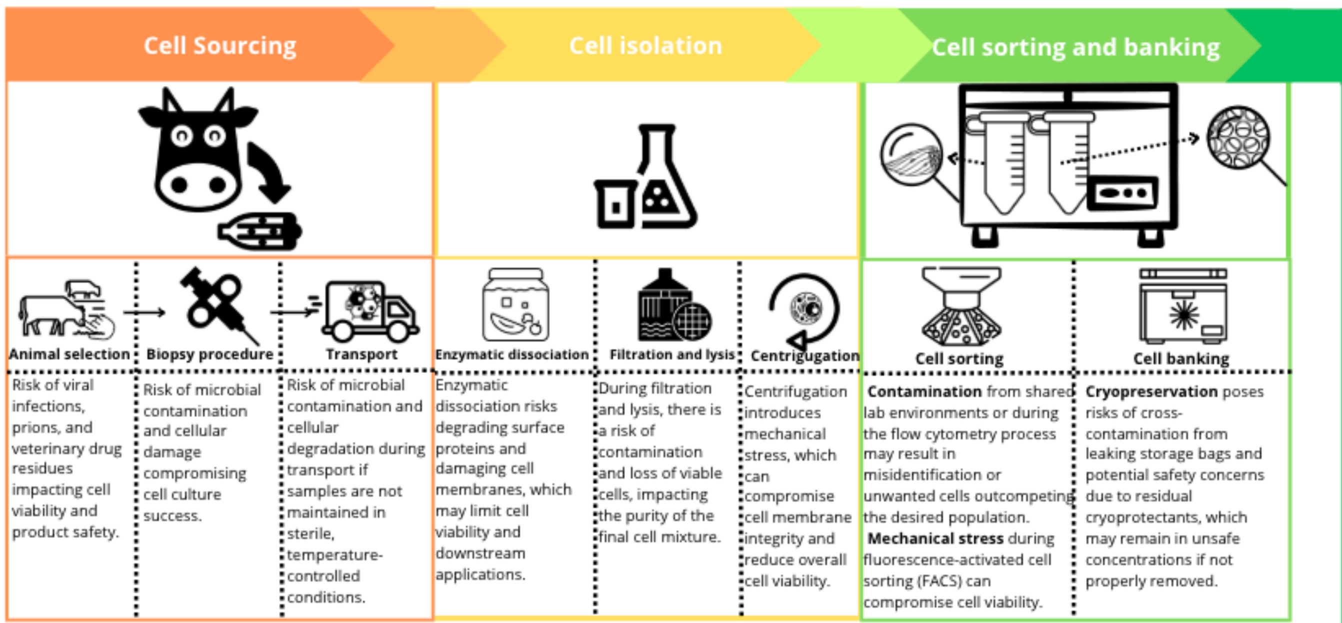
To support these strategies, structured methods and procedures are necessary. These include comprehensive risk assessments and consultations with experts, ensuring that safety is an integral part of the product's development. An example of this approach can be found in the Stage-Gate Model used for nanomaterials development. At each gate, decision-making is guided by specific criteria, and if these criteria are not fulfilled, the nanomaterial must return to the design table for further refinement (Robay, 2018).

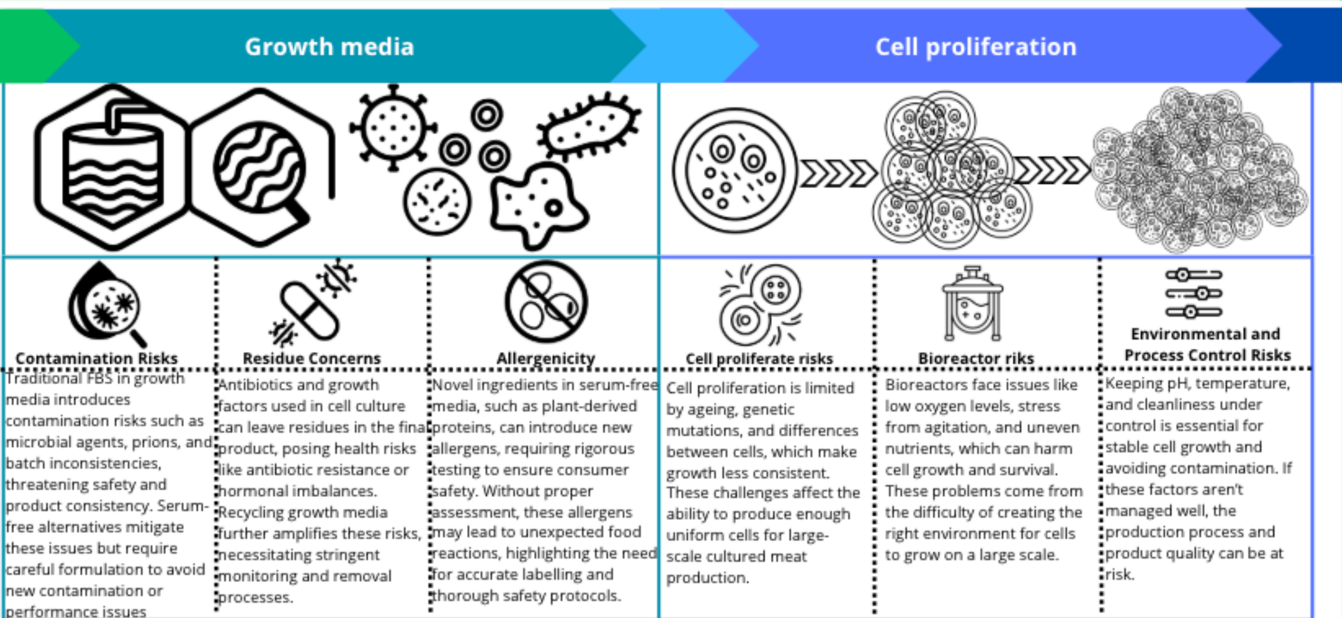
In the context of cultivated meat, four key risks can be observed: biological risks (e.g. contamination or pathogenic development), chemical risks (e.g. growth media, additives), process risks (equipment failure or deviations in environmental conditions) and lastly, consumer safety risks (allergens or long-term effects). The production process of CM involves many stakeholders. The main groups working towards getting the first cultivated meat products approved in the EU include EFSA, cultivated meat producers, production process designers, investors, consumers, research and development companies, and labs. These groups are expected to collaborate while also focusing on their specific roles to address safety and ethical concerns. By working together in this way, they can help make the approval process smoother and bring cultivated meat to the EU market more efficiently.

SbD provides a structured and iterative approach to addressing these risks. Integrating safety considerations at every stage, using risk assessments, and engaging multidisciplinary expertise ensures that risks are thoroughly evaluated and mitigated. Moreover, the alignment of SbD strategies with regulatory requirements could facilitate smoother approval processes, enabling cultivated meat to be safely and sustainably introduced to the European market. Through these efforts, Safe-by-Design serves as a cornerstone for the responsible development of cultivated meat, prioritising both consumer safety and technological innovation.

6. Identifying Risks and Mitigation Strategies in the CM Production Process

The risks associated with the production process of cm will be identified, and potential mitigation methods for these risks will be researched and discussed. The research will be conducted from cell sourcing up until the product formation. Each stage in the production chain is analysed to identify risks that must be managed to promote safety, scalability, and market acceptance.


Cell Sourcing
Cell isolation
Cell sorting and banking
Animal selection
Biopsy procedure
Transport
Enzymatic dissociation
Filtration and lysis
Centrigugation
Cell sorting
Cell banking
Risk of viral infections, prions, and veterinary drug residues impacting cell viability and product safety.
Risk of microbial contamination and cellular damage compromising cell culture success.
Risk of microbial contamination and cellular degradation during transport if samples are not maintained in sterile, temperature-controlled conditions.
Enzymatic dissociation risks degrading surface proteins and damaging cell membranes, which may limit cell viability and downstream applications.
During filtration and lysis, there is a risk of contamination and loss of viable cells, impacting the purity of the final cell mixture.
Centrifugation introduces mechanical stress, which can compromise cell membrane integrity and reduce overall cell viability.
Contamination from shared lab environments or during the flow cytometry process may result in misidentification or unwanted cells outcompeting the desired population.
Mechanical stress during fluorescence-activated cell sorting (FACS) can compromise cell viability.
Cryopreservation poses risks of cross-contamination from leaking storage bags and potential safety concerns due to residual cryoprotectants, which may remain in unsafe concentrations if not properly removed.


Growth media
Cell proliferation
Contamination Risks
Residue Concerns
Allergenicity
Cell proliferate risks
Bioreactor riks
Environmental and Process Control Risks
Traditional FBS in growth media introduces contamination risks such as microbial agents, prions, and batch inconsistencies, threatening safety and product consistency. Serum-free alternatives mitigate these issues but require careful formulation to avoid new contamination or performance issues
Antibiotics and growth factors used in cell culture can leave residues in the final product, posing health risks like antibiotic resistance or hormonal imbalances. Recycling growth media further amplifies these risks, necessitating stringent monitoring and removal processes.
Novel ingredients in serum-free media, such as plant-derived proteins, can introduce new allergens, requiring rigorous testing to ensure consumer safety. Without proper assessment, these allergens may lead to unexpected food reactions, highlighting the need for accurate labelling and thorough safety protocols.
Cell proliferation is limited by ageing, genetic mutations, and differences between cells, which make growth less consistent. These challenges affect the ability to produce enough uniform cells for large-scale cultured meat production.
Bioreactors face issues like low oxygen levels, stress from agitation, and uneven nutrients, which can harm cell growth and survival. These problems come from the difficulty of creating the right environment for cells to grow on a large scale.
Keeping pH, temperature, and cleanliness under control is essential for stable cell growth and avoiding contamination. If these factors aren't managed well, the production process and product quality can be at risk.

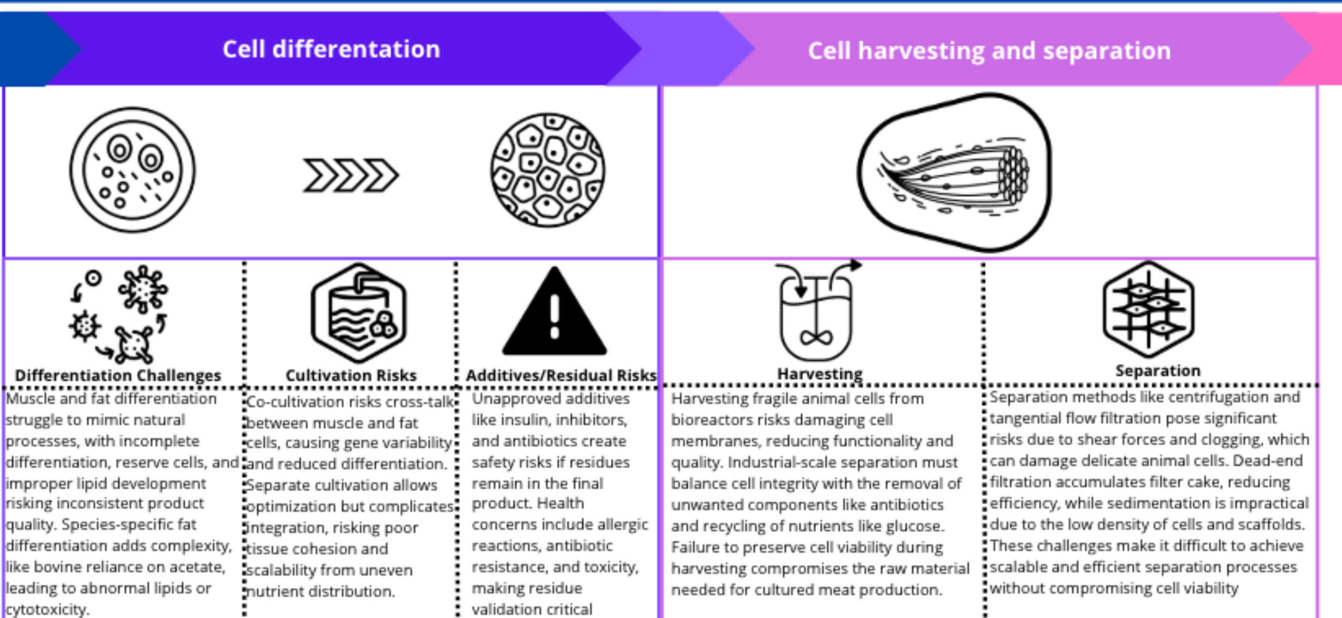

Cell differentation
Cell harvesting and separation
Differentiation Challenges
Cultivation Risks
Additives/Residual Risks
Harvesting
Separation
Muscle and fat differentiation struggle to mimic natural processes, with incomplete differentiation, reserve cells, and improper lipid development risking inconsistent product quality. Species-specific fat differentiation adds complexity, like bovine reliance on acetate, leading to abnormal lipids or cytotoxicity.
Co-cultivation risks cross-talk between muscle and fat cells, causing gene variability and reduced differentiation. Separate cultivation allows optimization but complicates integration, risking poor tissue cohesion and scalability from uneven nutrient distribution.
Unapproved additives like insulin, inhibitors, and antibiotics create safety risks if residues remain in the final product. Health concerns include allergic reactions, antibiotic resistance, and toxicity, making residue validation critical
Harvesting fragile animal cells from bioreactors risks damaging cell membranes, reducing functionality and quality. Industrial-scale separation must balance cell integrity with the removal of unwanted components like antibiotics and recycling of nutrients like glucose. Failure to preserve cell viability during harvesting compromises the raw material needed for cultured meat production.
Separation methods like centrifugation and tangential flow filtration pose significant risks due to shear forces and clogging, which can damage delicate animal cells. Dead-end filtration accumulates filter cake, reducing efficiency, while sedimentation is impractical due to the low density of cells and scaffolds. These challenges make it difficult to achieve scalable and efficient separation processes without compromising cell viability
Product formation, Packaging, distribution storage and consumer consumption

Figure 2: Overview of Risks in the Cultivated Meat Production Process The flowchart illustrates the cultivated meat production process, highlighting key risks at each stage: cell sourcing, isolation, sorting and banking, growth media, cell proliferation, differentiation, and harvesting/separation. Icons used in the figure were obtained from The Noun Project (n.d.).

6.1. Cell sourcing

For cultivated meat production, animal cells must be sourced. During this research, the focus will lie on bovine cells. Ensuring the animal's health, ethical considerations, and the biopsy methods employed are all critical to obtaining high-quality cells while minimising risks to the final product (Lanzoni et al., 2024).

6.1.1. Animal selection

To ensure the best possible quality of cells, several key factors need to be considered. The primary goal is to extract the largest number of the desired cell types from a single animal. The selection of the animal is not random; it is influenced by factors such as the animal's age, sex, living conditions, and genetic characteristics (Lanzoni et al., 2024). For instance, older animals tend to have fewer satellite cells, and these cells lose their differentiation potential more rapidly. Male animals often yield a higher concentration of stem cells due to the effects of testosterone, while animals raised in extensive farming (more 'relaxed' and focused on animal welfare) conditions, which include different dietary practices, may provide better results compared to those from intensive farming environments (Lanzoni et al., 2024). Although this step may not appear highly technical, it is critical to address these considerations early in cultivated meat development to ensure success.

Additionally, there are also risks related to viral infections, such as hepatitis or bovine leukaemia virus, especially if the source animal was infected. It is still unclear whether virally infected cells can survive in culture and pose a risk to the final product or not, and is therefore still being researched at this time. (Lanzoni et al., 2024).

Another possible infection can be that of prions. Prions are infectious agents that are known to cause several neurodegenerative diseases in both humans and animals, such as Creutzfeldt-Jakob Disease (CJD) in humans and bovine spongiform encephalopathy (BSE) in cattle. These diseases are associated with abnormal proteins called prions. Prions accumulate in specific tissues, including the brain, spinal cord, lymphoid tissues, and the enteric nervous system. When it comes to the production of CM, prions pose a significant food safety risk. Consuming meat from animals infected with prions could potentially lead to variant CJD in humans. Therefore, it is essential to avoid sourcing cells from tissues known to harbour prions, such as the brain and spinal cord, and to ensure that the source animals are certified free from BSE. Since the mechanisms of prion transmission and tissue distribution remain not fully understood, completely eliminating this risk remains challenging (Ong et al., 2021). To avoid these infection risks, it is important to do a thorough screening of the source animal for possible infections before taking the biopsy. To make sure that no infected cell will be used to start a cell culture. It will still be important to check biopsied cells for infections or other possible pathogens that may be present.

Another concern is contamination with veterinary drugs like antibiotics, which might be present in the tissue of the animal and potentially persist in CM, where it poses as a health risk

to consumers if concentrations exceed safe limits. These risks can be effectively managed by implementing rigorous testing protocols for drug residues (Lanzoni et al., 2024).

Another method of cell collection involves isolating large quantities of differentiated cells from animals post-slaughter. However, this approach presents significant challenges It requires the slaughter of more animals, contradicting the core principle of cruelty-free meat production. Additionally, differentiated cells have limited ex vivo proliferation capacity, making them unsuitable for long-term production (Kirsch et al., 2023). These considerations, summarised in table 2, underline the importance of carefully selecting animals that are not only healthy but also optimised for cell proliferation, ensuring that the harvested cells are viable and safe for large-scale cultivated meat production.

Table 2: Risk mitigation strategies during animal selection for cell sourcing

RISKS	MITIGATION STRATEGIES
Suboptimal cell availability, quality, and differentiation potential due to animal factors	Select younger, male animals raised in extensive farming environments, as they tend to provide a higher quantity and quality of satellite cells and stem cells due to favourable physiological and welfare conditions.
Risk of viral infections and pathogens	Perform thorough pre-biopsy screening to ensure the animal is virus-free. Post-biopsy, test cells for viral contamination and discard infected samples.
Risk of prion contamination	Avoid sourcing cells from high-risk tissues. Only use animals certified free of prion-related diseases. Employ stringent screening protocols to test for prion presence.
Contamination with veterinary drugs	Conduct rigorous testing for veterinary drug residues in biopsied tissues. Ensure source animals have minimal or no exposure to antibiotics through monitored farming practices.
Use of differentiated cells from slaughtered animals with limited proliferation capacity	Focus on undifferentiated stem or satellite cells, which have greater ex vivo proliferation potential. Prioritize biopsy-based sourcing rather than post-slaughter collection to align with cruelty-free goals.

6.1.2 Biopsy procedure

When extracting cells from living animals for cultured meat production, two main biopsy methods are used for the collection of tissue: a needle biopsy and one requiring a small incision. Considerations of animal welfare can influence the choice of method, as needle biopsies are generally less invasive but also yield fewer cells (± 0.5 grams), which may result in an insufficient collection of cells. Also, the fact that the biopsy is 'blind' increases the likelihood of collecting unwanted cells (Melzener et al., 2020). All risks and mitigation strategies for the biopsy step of cell souring are summarised in Table 3.

A biopsy done with an incision is more invasive, but there is more control over the nature of the collected sample and more cells are obtained (±15 grams). To optimise animal welfare during the biopsy, the animal should be placed in a treatment cage, sedated, and administered

local anaesthesia. This way, the animal will experience as minimal stress as possible, and the veterinarian doing the procedure will be safe. Afterwards, the animal can be treated with a painkiller to minimise discomfort. Also limiting the procedure to a maximum of four biopsies per session is recommended, allowing for sufficient recovery time between sessions (Melzener et al., 2020).

One of the concerns is cellular damage during the biopsy procedure, which can reduce the cells' ability to proliferate and grow in culture. The handling of the needle can introduce mechanical stress, leading to cellular damage and reduced ability to proliferate and grow in culture. Also, microbial contamination poses a constant risk, particularly from microorganisms found in the environment or on the skin and hair of the animal, which can contaminate the tissue during sampling (Soccol et al., 2024). To mitigate this risk, the biopsy site should undergo trichotomy (hair removal), thorough cleaning with disinfectant, and inspection for signs of infection. Also, the air in the environment where the biopsy is done can be contaminated because of the farming conditions (Sogore et al., 2024), which makes it important to do the biopsy in a sterile environment and quickly isolate the sample from the surrounding air.

The cells need to be transported from the biopsy site to the lab, it is important to maintain their integrity and viability during transport. Therefore, the biopsy sample should be placed in a sterile container filled with a buffered transport medium, such as phosphate-buffered saline (PBS), which preserves the physiological pH and osmotic balance of the cells during transit. To prevent microbial contamination, antibiotics and antifungal agents are often added to the transport medium. Additionally, the appropriate temperature conditions need to be maintained throughout the transport process to ensure the cells remain viable. The sample should be packaged in a leak-proof container to protect against contamination and ensure safe handling. Lastly, timely transport is essential to minimise the time between sample collection and processing, making sure the quality of the cells is preserved for further use in the lab (Robinson, 2024).

Table 3: Risk mitigation strategies during and post biopsie.

RISKS	MITIGATION STRATEGIES
Insufficient cell yield, leading to an inadequate sample.	Optimise biopsy techniques to maximise yield, such as selecting incision biopsies method for larger tissue samples
Unwanted cells collected, reducing culture efficiency.	Opt for incision biopsies for better control over the sample and selection of desired cells.
Invasiveness of the biopsy causing harm and stress to the animal. And potential overuse of biopsies	Ensure proper technique to minimise harm. To reduce stress, sedate the animal and provide it with painkillers. Limit biopsies to a maximum of four biopsies per session.
Cellular damage reducing proliferation and growth capacity.	Minimise mechanical stress during biopsy procedure. Training in advanced biopsy techniques to reduce risks.
Microbial contamination from skin, hair, or the environment.	Perform trichotomy, disinfect the biopsy site thoroughly, and conduct the procedure in a sterile environment.

Airborne contamination due to farming conditions.	Conduct biopsies in sterile facilities or cleanroom environments to reduce exposure to airborne contaminants.
Risk during biopsie transport	Use sterile, leak-proof containers filled with PBS supplemented with antibiotics and antifungals, maintain controlled temperature conditions, and ensure rapid transport.

6.1.3 Cell types

Many types of cells can be sourced from animals for cultivated meat production, all containing their own risk and challenges, as shown in Table 4 . Different types of cells can be used: adult stem cells (ASCs), embryonic stem cells (ESCs), and induced pluripotent cells (iPSCs), each offering distinct advantages and limitations. The selection of initial cell types plays a crucial role in regulatory considerations and in the scientific development of the technology as well. Regardless of the specific approach used for cultured meat production, a thorough understanding and precise control of stem cells are essential throughout the process.

ASCs are muscle stem cells, my satellite cells, and mesenchymal stem cells. These cells are easily obtained through a needle biopsy and are not classified as genetically modified organisms. They also have established differentiation protocols available, making them accessible for production. The proliferation capacity of the cells is limited, so there is a need for frequent starter samples, which call for frequent biopsies (Martins et al., 2024). This phenomenon represents a significant bottleneck in the large-scale production of cultured meat, as it limits the number of times cells can be expanded. Immortalisation can be achieved by introducing genes like TERT or oncogenes such as c-MYC, which can extend telomeres or promote continuous cell division. However, this process presents regulatory and safety concerns, as GMOs often face consumer resistance and regulatory barriers. Additionally, the risk of tumour formation is heightened when cells are genetically altered to bypass their natural growth limits (Kirsch et al., 2023). Somatic cells can be reprogrammed through genetic manipulation, chemical methods, or spontaneous mutations. However, this process may result in unintended genetic changes, such as genetic drift, which can pose safety risks during growth, proliferation, and differentiation in the final product. Genetic stability can be monitored through chromosomal analyses and the use of freshly preserved starter cells (Gu et al., 2023).

ESCs offer a high proliferation capacity and the ability to differentiate into various cell types, which makes them theoretically ideal for large-scale production. However, their use raises ethical concerns since ESCs are derived from embryos, which leads to controversy and resistance among consumers and regulators. Another issue is that ESCs can accumulate mutations over time, posing risks to product safety and consistency (Benny et al., 2022). Another disadvantage is the lack of clear protocols for differentiation in livestock species.

Induced pluripotent stem cells could be an alternative to ESCs by reprogramming adult somatic cells back into a pluripotent state, which allows them to differentiate into various cell types. However, iPSCs, like immortalised cells, often involve genetic modification, which raises more complex regulatory and safety issues. iPSCs are also subject to genetic instability,

which can cause cells to lose their ability to proliferate or differentiate correctly over time (Zhang et al., 2021).

Chemically induced pluripotent stem cells are a newer and promising alternative, created through chemical reprogramming without the need for genetic modification. This approach could mitigate some of the concerns associated with GMOs. However, the technology is still in its early stages and is not yet validated for commercial-scale production (Kirsch et al., 2023).

Each one of these cell types presents different opportunities and challenges, from regulatory hurdles around genetic modification to overcoming a biological limitation like the Hayflick limit when normal cells stop dividing due to senescence (Kirsch et al., 2023). As cells near this limit, changes in cell structure and broad shifts in gene expression happen. Immortalisation strategies can potentially extend the life span of cells for large-scale production, but they come with ethical and safety concerns that must be carefully managed to ensure consumer safety. The ideal cell line would be easy to isolate, efficiently proliferate in cost-effective media, respond well to straightforward differentiation protocols, and remain resilient to environmental changes (Martins et al., 2024).

Table 4: Limitations and risks of the discussed cell types and the possible mitigation strategies.

	Limitations and Risks	Mitigation Strategies
ASCs	- Limited proliferation capacity, requiring frequent biopsies. - Immortalisation processes carry risks of tumour formation and regulatory barriers. - Risk of genetic drift during cell manipulation.	- Use advanced immortalisation techniques while ensuring genetic stability through regular chromosomal analyses. - Maintain fresh starter cells to minimise drift. - Research non-invasive methods to increase proliferation potential.
ESCs	- Ethical concerns - Accumulation of mutations, compromising safety and consistency. - Lack of established differentiation protocols.	- Focus on alternatives (e.g., iPSCs) to address ethical concerns. - Develop advanced differentiation protocols tailored to livestock cells.
iPSCs	- Often require genetic modification, raising regulatory and consumer acceptance issues. - Genetic instability over time reduces ability to proliferate or differentiate effectively. - Early-stage technology not yet validated for large-scale production.	- Pursue chemically induced reprogramming to avoid genetic modification. - Implement rigorous testing and monitoring for genetic stability during culture expansion. - Encourage further research and development to validate scalability.
Chemically Induced Pluripotent Stem Cells	- Early-stage development with no validation for commercial-scale production. - Limited data on long-term stability and differentiation efficiency.	- Invest in research for optimisation and scalability. - Conduct comparative studies with iPSCs to benchmark effectiveness and stability. - Ensure rigorous quality control during early testing phases.

6.2. Cell culture development

Cell culture development is a complex process central to the production of CM. It involves the growth and propagation of the collected bovine cells in a controlled laboratory environment. These cells will be grown in media containing essential nutrients, growth factors, and hormones to promote their growth and multiplication. The cell culture process consists of multiple stages, each associated with specific risks that must be addressed to ensure consumer safety, as shown in Table 5 below.

Table 5: Risks and Mitigation Strategies in Cell Culture Development for Cultivated Meat Production.

Step	Risks	Mitigation Strategies
Cell Isolation, Dissociation, and Selection	- Mechanical damage degrades surface proteins or damages cell membrane. - Contamination from improper handling or non-sterile environments. - Yield loss due to enzymatic inefficiencies or overprocessing.	- Use gentler dissociation agents like Accutase or non-enzymatic solutions to preserve cell integrity. - Employ rigorous sterilisation protocols for instruments and workspaces.
Cell Growth	- Inconsistent nutrient availability and oxygen levels within culture vessels may lead to variable cell growth - Risk of contamination from media components or handling. - Nutrient imbalance leading to cell death or malfunction.	- Use specialised culture media tailored to the specific cell type. - Incorporate routine contamination testing and adjust nutrient compositions as needed.
Cell Sorting and Banking	- Cross-contamination of cell populations, particularly in shared lab environments. - Mechanical damage during sorting processes (e.g., FACS). - Risks associated with improper cryopreservation, such as cryoprotectant residues or bag leakage.	- Maintain isolated storage areas for cell banking and sorting to prevent contamination. - Optimise cryopreservation protocols, including the use of vapour-phase liquid nitrogen to minimise pathogen transfer. - Use flow cytometry for frequent quality control checks.
General Risks in Cell Culture	- Microbial contamination from bacteria, fungi, or viruses. - Variability in cell culture media due to batch inconsistencies. - Cross-contamination due to improper handling	- Implement routine microbiological testing across all stages. - Transition to chemically defined, animal component-free media to improve consistency. - Separate laboratory for each cell type and strictly enforced laboratory protocols.
Specific Contamination Risks	- Mycoplasma contamination often goes unnoticed, altering cell behaviour and results. - Viral contamination poses greater risks due to integration into host cell genomes, impacting cell line stability.	- Follow strict laboratory protocols to prevent contamination. - Conduct regular testing using electron microscopy for example to confirm virus-free status. - Maintain sterile environments during all stages of cell culture development.

Environmental Factors	- Fluctuations in nutrient gradients, pH, and oxygen availability lead to inconsistent cell growth and behaviour. - Batch variability in serum or supplements. - Overuse of antibiotics can alter cell metabolism and gene expression. - Genetic drift in continuous cell lines compromises integrity. - Enzymatic detachment during passaging may damage cells.	- Maintain a controlled environment to minimise fluctuations and monitor often. - Use chemically defined, standardised culture media. - Limit antibiotic use; emphasise sterile techniques. - Monitor genetic stability rigorously. - Use careful, precise passaging techniques to minimise cell damage.

6.2.1 Cell isolation; dissociation, and selection

The first step in cell culture development involves isolating and dissociating the cells collected from biopsies. The biopsy sample must be processed to isolate the individual cells. This can be done by mechanical methods, such as mincing the tissue, or by enzymatic methods, using enzymes to separate the cells from each other (Ong et al., 2021).

Various risks are involved during this isolation process, which can compromise the culture's yield and quality. The primary challenge in cell isolation is extracting cells to obtain high yields of high-quality target cells. This often involves enzymatic dissociation, filtration, and differential centrifugation. To start, the extracellular matrix (ECM), which binds the cells together, is broken down using enzymes such as trypsin and collagenase. Additionally, an agent like EDTA is also used to bind calcium ions, which are necessary for cell adhesion. Once these treatments are applied, gentle agitation helps to separate the tissue into individual, living cells (Alberts et al., 2002).

Then, the remaining cell source is filtered, and the cells are incubated in an ammonium-chloride-potassium (ACK) erythrocyte lysis buffer (Dohmen et al., 2022). This buffer lyses the red blood cells from the wanted cell mixture (AAT Bioquest, n.d.), leaving only the wanted cells at this stage. In between these steps, centrifugation is done as well (Ding et al., 2018). Mechanical damage during dissociation, such as the use of trypsin, can degrade surface proteins necessary for further cell analysis or purification steps, and it can also damage the cell membrane. This may limit the ability to use techniques such as flow cytometry for cell separation or characterisation. The use of alternative gentler dissociation agents like Accutase or non-enzymatic solutions is recommended to minimise such risks, preserving surface epitopes and ensuring the integrity of cells (Weiskirchen et al., 2023). Once the cells have been successfully isolated and purified, the next phase focuses on promoting their growth and expansion in a controlled environment.

6.2.2. Cell growth

After isolating and purifying the desired cell types, the next step is developing cell cultures. This can result in either a primary cell culture or the establishment of a cell line. The success of this transition from isolated cells to a stable and functional cell culture depends on various

factors, including the choice of appropriate culture media, environmental conditions, and techniques to support cell growth, proliferation, and differentiation.

In the initial phase, isolated cells are cultured in specialised media containing nutrients, growth factors, and other supplements essential for sustaining cell viability and promoting their expansion. This phase is critical, as the cells must adapt to the in vitro environment, which can be very different from their native tissue conditions. Challenges such as selecting the correct media, preventing contamination, and ensuring proper nutrient balance are key to preventing cell death or malfunction (Weiskirchen et al., 2023).

6.2.3. Cell sorting and banking

As the cells grow and expand, the next step involves sorting specific cell types and preserving them for future use, ensuring consistency and scalability in the production process. This is done in a cell culture development flask, so the cells have something to adhere to, which stimulates proliferation. Once the culture expands, cells are sorted to isolate satellite muscle cells and fat progenitor cells (or other target cells). The sorting can be done with FACS (fluorescence-activated cell Sorting). In this method, antibodies specific to different cell types are used to label the cells for sorting. The cells will then be processed through a flow cytometer, which sorts them based on size and other characteristics. Only live cells needed for the remainder of the process are selected and separated (Song et al., 2022). Flow cemetery can be used while the batch expands for frequent batch controls if only the desired cells are present.

This process carries a risk of cross-contamination and mechanical damage, so it is of great importance that it is properly handled (Weiskirchen et al., 2023). Misidentification or cross-contamination of cells is also a risk, especially in shared lab environments where multiple cell lines are handled. These errors can allow contaminant cells to outcompete the intended population, leading to unreliable results (Martins et al., 2024).

Cell banking is done for the storage of cells before using them in the following steps. The process is a crucial step in ensuring the stability of cell lines and the consistency of cell-based products, in this case, cultured meat This process reduces the need to repeatedly source cells from animals, providing a stable, reliable, and more ethical way to obtain cells. During this procedure, cells (either isolated primary cells or those further developed) are selected, validated, and frozen in small batches. These batches can be thawed, revalidated, and expanded as needed for product generation. The preservation technique often involves vitrification, a rapid freezing process that helps reduce the risk of intracellular crystallisation, which can damage cells. Additionally, the cells are stored at very low temperatures, frequently under liquid nitrogen, with the use of cryoprotectants to protect cell viability and functionality (Ong et al., 2021).

However, this preservation process presents specific risks, such as contamination and residual chemicals. For instance, leakage of cryopreservation bags during storage in liquid nitrogen poses a cross-contamination risk. Pathogens may transfer between stored cells, even with specialised freezing bags. Additionally, cryoprotectants, while essential for cell protection during freezing, may present safety issues if they remain in the final product in unsafe concentrations. Certain cryoprotectants, like insulin, sorbitol, and dimethyl sulfoxide, are

approved for food use at specific levels. However, the introduction of novel cryoprotectants requires rigorous safety evaluation (Ong et al., 2021).

Effective mitigation strategies are crucial to minimise the risks associated with cryopreservation and cryoprotectant use. To prevent cross-contamination, cell banks are often stored in the vapour phase of liquid nitrogen rather than the liquid phase, thereby minimising pathogen transfer potential. Furthermore, the washing or dilution of cryoprotectants during product development is expected to reduce their concentration to safe levels in the final product, this needs to be tracked through the production stages. In cases where novel cryoprotectants are used, companies may need to develop and validate specific analytical tests to detect any residuals or byproducts. Conventional methods, such as mass spectrometry and chromatography, along with bioassays designed to detect chemical residues in traditional meat, may require adaptation for use with cell-cultured meat products (Ong et al., 2021).

6.2.4 General risks in cell culture development

Cell culture development is technically demanding and expensive, particularly when isolating specific cell types from heterogeneous tissue (Gu et al., 2023). One of the key risks during cell isolation is the potential for contamination, including microbiological contamination from bacteria, fungi, or viruses. This can occur at any stage due to improper handling, unsterile instruments, or exposure to non-sterile environments (Weiskirchen et al., 2023).

Cross-contamination poses a significant risk in all the laboratories handling multiple cell types or working with various biological samples. In cell culture, cross-contamination refers to the accidental introduction of cells from one culture into another. This can occur because of simple mistakes, such as using the same pipette or medium for different cultures without adequate sterilisation or through accidental spread of cells via droplets or splashes. Cross-contamination is particularly concerning because it often goes unnoticed; cells from one culture can overgrow or outcompete the intended cell line, altering the experimental results. Once contaminated, distinguishing between the original and contaminant cells can be challenging, especially in closely related cell types, potentially leading to inaccurate data and false conclusions (Food Standards Agency, 2023).

6.2.5 Specific Contamination Risks

There is also mycoplasma contamination, which refers to an infection of cell cultures by the mycoplasma bacteria. These are tiny organisms that lack a cell wall and are resistant to many common antibiotics. This type of contamination is difficult to detect because it does not cause visible changes in the culture, such as cloudiness, which typically signals bacterial contamination. They can spread through laboratory equipment, reagents, or even air and human contact. Mycoplasma competes with the host cells for nutrients and interferes with cellular functions like DNA, RNA, and protein synthesis. This can lead to altered cell behaviour, misleading experimental results, and possibly even the loss of the entire cell culture. Because the contamination is dangerous, it often goes unnoticed and can spread across labs. Where it can impact the reliability of research. Eliminating mycoplasma contamination can be challenging; it requires specific treatments and careful monitoring. The prevention of this kind

of contamination is essential and can be done by setting strict laboratory protocols to ensure the integrity of the cell cultures (Weiskirchen et al., 2023).

Viral contamination in cell cultures poses a more significant risk compared to mycoplasma infections, as viruses are harder to detect and are not easy to treat once the culture is infected. Certain viruses can integrate their genetic material into the host cells, leading to the continuous production of viral particles. These particles can then spread to other cell lines. This presents a potential health risk to researchers and has serious implications for the biological safety classification of the affected cell line. Detecting viral contaminants is complex and often requires highly specialised techniques. Standard methods like electron microscopy are used to visually confirm the presence of viruses. This provides high-resolution images that reveal the actual infection status and can help classify the virus based on its morphology and size (Weiskirchen et al., 2023). Regular monitoring of cell cultures for these viral particles would be essential during cell culture development.

6.2.6. Environmental factors in the cell culture process

Other than direct contamination, environmental factors also play a crucial role in ensuring successful cell culture development. These factors could influence both cell health and overall process efficiency. Nutrient gradients, pH levels, and oxygen availability often fluctuate within culture vessels, which can impact cell viability and growth rates. For instance, cells in different areas of the flask may receive uneven nutrients or oxygen, resulting in inconsistent growth and behaviour within the culture. These inconsistencies are often exacerbated by variability in the quality or composition of cell culture media. For instance, the quality and composition of serum or other supplements can vary between batches, introducing additional variability. Overuse of antibiotics to prevent contamination is another risk factor; while antibiotics help maintain sterility, they can alter cell metabolism and gene expression, affecting cellular behaviour and experimental outcomes (Weiskirchen et al., 2023).

For primary cell cultures, which are typically derived directly from tissue, the aim is to grow the cells without altering their original characteristics. These cells can be difficult to maintain as they have a finite lifespan and often exhibit slower growth. To create continuous cell lines, cells can be immortalised through genetic manipulation or, less commonly, spontaneous mutations. This enables indefinite proliferation (Gu et al., 2023). Continuous cell lines require rigorous monitoring for genetic drift and stability, as any alterations during the proliferation process could compromise the integrity of the culture and lead to unwanted cell behaviours or product contamination (Martins et al., 2024). As cells grow and proliferate, they require careful passaging to maintain optimal density and viability. In the case of adherent cells, passaging typically involves enzymatic detachment. With this type of detachment, agents like trypsin are used to release the cells from the surface they are attached to. Ensuring that this process is performed carefully is crucial to avoid damaging the cells or altering their characteristics (Weiskirchen et al., 2023). On the other hand, suspension cells can grow freely in the medium and often require less manipulation during the passaging process. Developing a robust cell line, whether for research or industrial purposes, requires continuous monitoring to ensure that the cells keep their desired properties over time. Inconsistent handling, environmental fluctuations, or contamination can lead to significant variations in cell

behaviour. Therefore, it is essential to implement stringent quality control measures throughout the cell culture and cell line development stages (Martins et al., 2024).

Lastly, the use of animal-derived reagents, such as fetal bovine serum, introduces variability and contamination risks from viruses, prions, or bacteria. Such risks demand rigorous quality control and characterisation to ensure contamination-free production of lab-grown meat (Weiskirchen et al., 2023). The development of chemically defined, animal component-free media is essential to mitigate these risks and enhance the reproducibility and safety of the final products (Martins et al, 2024). In conclusion, ensuring a clean, controlled, and well-characterised environment during cell isolation is critical to prevent contamination, maintain cell quality, and safeguard the value chain up to the end consumer. Regular testing for contaminants and adopting appropriate dissociation methods are essential strategies to minimise risks.

Cultivated meat production starts with the careful culturing of cells in a lab. Once they have reached the appropriate development stage, the cells are moved to an industrial setting where the process scales up significantly. At this point, the cells are placed into bioreactors, which provide a highly controlled environment that supports their rapid growth on a much larger scale. In these bioreactors, a specialised growth medium constantly supplies essential nutrients, growth factors, and other elements to keep the cells growing. This medium not only meets the cells' basic nutritional needs, like glucose, amino acids, and vitamins but also helps create ideal conditions for efficient large-scale production (Kirsch et al., 2023). Throughout this process, the cells divide and grow, forming the biomass needed for cultivated meat. However, moving from lab to industrial scale introduces several critical factors that need careful management: the growth media for cell nutrition, the bioreactors for maintaining a stable environment, and ensuring the cells remain stable for a consistent product. Each of these factors is essential for production, but they also bring specific challenges, especially concerning consumer safety and production stability (Chodkowska et al., 2022).

6.3. Growth media: Risks, ethical and safety concerns

During the cell proliferation phase, the cells grow in bioreactors containing media. Growth media is a very relevant topic and contains many aspects of safety; key findings are summarised in Table 6.

One of the main risks during the cell proliferation phase is the potential contamination of the growth medium. Traditionally, FBS has been used as a supplement in cell culture media due to its high concentration of growth factors. However, FBS has several associated risks, such as possible contamination with viruses or prions and notable ethical concerns due to its animal origin. The reliance on animal-derived serum not only introduces ethical concerns but also significant safety risks. For instance, FBS can introduce microbial agents like bacteria, fungi, or even viruses, which pose a risk during production and may also impact consumer safety if not properly managed. Additionally, the use of animal-based components conflicts with the ethical objectives of cultivated meat, which aims to reduce reliance on animal agriculture (Chodkowska et al., 2022). Also, the use of FBS comes with batch inconsistencies (Sogore et al., 2024).

In response to these concerns, there has been significant progress toward developing serum-free and chemically defined media as alternatives to FBS. These serum-free formulations aim to replicate the nutrient profile of FBS but use plant-based, recombinant, or synthetic components to avoid the risks linked to FBS. Developing these media is crucial not only for reducing production costs but also for aligning with the ethical objectives of cultivated meat, particularly reducing reliance on animal sources. However, transitioning to serum-free media presents certain challenges. These formulations must be carefully optimised to support the specific cell types used in production, which often requires custom tailoring of ingredients to ensure the cells receive the necessary signals for growth and differentiation (Martins et al., 2024). While serum-free media offer clear advantages, their development and implementation present unique challenges that must be addressed.

The transition to serum-free media in cultivated meat production presents ethical and economic benefits but also introduces specific risks that must be managed to ensure product safety and viability. A primary risk associated with serum-free media is the potential for inadequate growth and differentiation of muscle and fat cells, as serum naturally contains numerous growth factors and nutrients which are not fully replicated in serum-free formulations. Serum provides key signalling molecules essential for cell proliferation and differentiation; without these components, cells may struggle to reach the desired scale or quality in culture, affecting product consistency (O'Neill et al., 2020).

Additionally, serum-free media must carefully balance the inclusion of recombinant growth factors to maintain cell health without introducing contaminants or residues that could pose food safety risks (Ong et al., 2021). Furthermore, serum-free formulations may lack the robustness of traditional serum in protecting cells from environmental fluctuations, such as temperature and pH changes during bioreactor proliferation (Gu et al., 2023). These collective risks highlight the importance of rigorous testing and optimisation of serum-free media to support large-scale production while meeting regulatory and safety standards

Allergenicity is a significant concern with components in growth media, particularly in serum-free formulations. Novel ingredients in serum-free media, such as recombinant proteins and plant-derived molecules, may introduce new allergens or increase cross-reactivity with existing allergens (Chodkowska et al., 2022). Specifically, proteins derived from plants pose potential allergen risks. If new proteins that are absent in traditional meat are present in cultured meat, they could raise allergenicity, as many food allergens are glycosylated proteins. To minimise allergen risks, the protein structures and amino acid sequences should be cross-referenced with known allergens using specialised databases. When homology with known allergens is found, further allergenicity tests are recommended, including in vitro digestive stability tests, cell line assays, rodent assays, IgE testing using human serum, skin prick testing, and controlled food challenge studies. Clinical allergenicity studies, which carry the risk of severe reactions, are typically avoided and only conducted if prior in vitro tests show no indication of allergenic potential. Furthermore, the impact of processing methods on potential allergenicity in cultured meat should be considered, and if allergens are identified, appropriate labelling is necessary to inform consumers (Gu et al., 2023).

Another critical safety issue with growth media is the use of antibiotics and antimicrobials to prevent contamination during cell culture. Antibiotics are frequently employed to control bacterial contamination; however, their overuse may result in antibiotic residues in the final product, potentially increasing the risk of antibiotic resistance in consumers (Ong et al., 2021).

Each component of the cell-culture media has the potential to persist in the final product, warranting distinct evaluation of media residues and byproducts. Introducing novel ingredients or using existing components at higher concentrations than in conventional meat may lead to additional safety concerns. For instance, the use of wheat gluten as a hydrolysate could introduce allergens into the product. In cultured meat production, signalling molecules like growth factors, which may be naturally produced or stimulated through genetic engineering, are often necessary. While these growth factors and hormones are essential for cellular function, excessive consumption may lead to health imbalances. Regulatory bodies in regions such as the European Union have restricted hormone use in conventional farming due to health risks from residues. Though some growth factors degrade during digestion or processing, others have shown resistance to degradation. Accumulation of growth media components is also a concern when media is recycled during production, as this can increase the concentration of certain molecules. Thus, identifying any health-related molecules, quantifying their residues in the final product, and comparing these levels with those in conventional foods is crucial to ensure consumer safety (Cai et al., 2023).

In vitro tests provide an efficient, resource-conserving method for safety assessment, reducing the need for animal testing. In vitro methods primarily assess individual ingredients rather than whole foods. Therefore, testing whole foods in vitro is more difficult because the processed samples may not represent the final product accurately. Tests like cytotoxicity, digestibility, and microbiome impact offer additional information, but they are not fully validated as alternatives to animal testing by regulators. For example, cytotoxicity tests can screen for harmful effects using gut cells, and digestibility tests check how stable food is under different conditions. Microbiome tests can help identify how residues or contaminants from growth media might affect gut health. However, more research is needed to develop reliable in vitro methods specifically for CM (Cai et al., 2023).

Table 6: Risks and mitigation strategies for media in cultivated meat production.

	Risks	Mitigation Strategies
FBS	- Batch variability and inconsistencies. - Contamination risks from viruses, prions, bacteria, and fungi. - Ethical concerns due to animal-derived origin.	- Transition to serum-free or chemicall defined media using plant-based, recombinan or synthetic components. Implement rigorou quality control testing for batch consistency. - Promote the development and adoption c animal-free formulations to align with ethica objectives.

Serum-Free Media	- Inadequate growth and differentiation of muscle and fat cells due to missing key nutrients and growth factors. - Lower robustness against environmental fluctuations	- Optimise media formulations to replicate th nutrient profile of FBS. - Include recombinant growth factors whil ensuring food safety through residue testing. - Conduct rigorous testing and optimisation c formulations to maintain consistent cell viabilit and differentiation.
Allergenicity	- Potential allergenicity from novel recombinant or plant-derived proteins. - Cross-reactivity with existing allergens due to glycosylated proteins.	- Compare amino acid sequences and protei structures with known allergens in allerge databases. - Conduct allergenicity testing - Label products appropriately if allergens ar identified.
Antibiotics and Antimicrobials	- Residues in the final product may increase antibiotic resistance in consumers. - Overuse during cell culture poses contamination risks.	- Reduce or eliminate the use of antibiotics in ce culture processes by developing robu contamination prevention protocols an sterilisation methods. - Test for residual antibiotics to ensure saf concentrations in the final product.
Growth Factors and Hormones	- Health risks from excessive residues. - Accumulation of residues in recycled media during production.	- Identify and quantify residual growth factors i the final product And compare residue level with conventional meat to ensure safety. - Evaluate digestion stability and degradation c growth factors.
In Vitro Safety Testing	- Limited ability to fully replicate final product conditions in vitro. - Difficulty assessing whole-food samples due to processing challenges.	- Use cytotoxicity, digestibility, and microbiom tests as complementary tools. - Develop improved in vitro assays specific t cultured meat. - Ensure in vitro tests align with regulator requirements.

6.4. Cell proliferation

In cultured meat production, achieving sufficient cell proliferation is critical to generating the biomass needed for large-scale production. However, balancing genetic stability, consistent growth, and cost-effectiveness remains a significant challenge, as shown in Table 7. The proliferation process must be optimised to occur under animal-free, scalable conditions. The selection of cell type influences the challenges encountered in maintaining this balance. Primary stem cells, for instance, experience a loss of proliferative and differentiation potential in culture over time. In contrast, pluripotent cells can support their growth but often require costly and complex media to do so. Because of this, it is important to understand the mechanisms driving cellular changes during proliferation; this can help develop strategies to extend expansion phases and support differentiation, ultimately increasing the yield and reducing variability and costs (Martins et al., 2024).

Achieving sustainable cell proliferation without animal-derived components (FBS) is still a challenge. In vivo, stem cells rely on complex signals from ECM components and neighbouring cells, which is difficult to replicate in vitro. nevertheless, serum-free media formulations that support the proliferation of key cell types, such as bovine satellite cells (SCs) and fibro/adipogenic progenitors (FAPs), have been developed as an important advancement in this field. Another key challenge is maintaining cells in a prolonged state of proliferation, as primary cells ultimately enter a senescent state during long-term culture. The relationship between cellular ageing and decreased proliferation highlights the need for interventions to extend the proliferative capacity of cells to meet the demands of large-scale meat production (Martins et al., 2024).

Genetic mutations and the Hayflick limit pose substantial risks to the stability of cell lines used in cultured meat. Cellular ageing involves various changes, including shifts in signalling pathway activity, metabolic adjustments, and mutations at both genetic and epigenetic levels, which accumulate over time. Prolonging cell viability might be possible through interventions, such as inhibiting the signalling pathway or targeting other pathways, although these approaches may encounter regulatory challenges (Martins et al., 2024). To mitigate this issue the development of stable cell lines capable of prolonged proliferation is critical, utilising genetic interventions to direct pluripotent stem cells into specific cell types, such as muscle or fat cells, to create desirable cultured meat properties can be an option (Cai et al., 2023).

Cellular heterogeneity within a culture is another critical consideration. Over time, subpopulations with varying growth rates and cellular states may emerge within SC and FAP cultures. These differences can impact growth consistency, leading to challenges in maintaining uniform cell behaviour in industrial applications. Without proper controls, heterogeneity can also arise within pluripotent cell cultures, affecting the proliferation phase and, thus, the quality of the final product (Martins et al., 2024). Moreover, contamination risks can exacerbate this heterogeneity, as foreign cell types or microorganisms may interfere with the primary culture. While antibiotics are frequently added to growth media to prevent bacterial contamination, their use is not ideal for large-scale production due to safety and regulatory concerns (Chodkowska et al., 2022).

Table 7: Key Risks and Mitigation Strategies for Cell Proliferation, Stability, and Media Optimisation in Cultivated Meat Production.

Risk Category	Specific Risks	Mitigation Strategies
Cell Proliferation and Aging	- Loss of proliferative and differentiation potential in primary stem cells over time. Entry into senescent state during prolonged culture. - Cellular ageing causing signalling shifts, metabolic changes, and genetic/epigenetic mutations.	- Develop strategies to extend expansion phases and support differentiation to increase yield and reduce variability. - Explore interventions targeting pathways to extend proliferative capacity. And inhibit cellular ageing
Consistency and Stability	- Emergence of cellular heterogeneity, including subpopulations with varying growth rates and states.	- Implement controls to maintain uniform cell behaviour during industrial-scale production.

	- Accumulation of mutations affecting genetic stability.	- Monitor and standardise culture conditions to reduce variability. - Develop stable cell lines capable of prolonged proliferation.
Media and Growth Environment	- Dependence on complex, costly media formulations for pluripotent cells. - Difficulty replicating complex in vivo ECM signals in vitro.	- Optimise serum-free media formulations for scalability and cost-effectiveness. - Develop advanced serum-free media formulations tailored to key cell types like SCs and FAPs.
Contamination Risks	- Contamination by foreign cells or microorganisms. Use of antibiotics for contamination control not ideal for large-scale production.	- Minimise reliance on antibiotics to mitigate contamination risks.

6.5. Bioreactors' challenges in scale-up

The cultivation of meat at a commercial scale faces challenges related to bioreactor design, including cell line stability, oxygen distribution, nutrient availability, regulation of environmental parameters, and the management of shear forces. Bioreactors must support large-scale cell proliferation while maintaining an in vivo-like environment that optimises cell growth, proliferation, and differentiation (Martins et al., 2024; Chodkowska et al., 2022).

A major limitation in bioreactors is the need for anchorage-dependent growth, which constrains cell surface area. Strategies such as microcarriers, cell aggregates, and hydrogel encapsulation are employed to overcome this. Edible microbeads, for example, enable suspension growth while eliminating the need for protease-based harvesting, simplifying downstream processing. However, microbeads may impact the final product's taste, texture, and colour. Stirred-tank bioreactors, widely used for their scalability, are often constrained by challenges such as oxygen gradients and hydrodynamic stress, which can adversely affect cell health and proliferation (Kirsch et al., 2023).

Oxygen levels in bioreactors must be controlled to sustain cell viability, particularly at high cell densities where oxygen deprivation can become an issue. Designs like air-lift and hollow-fibre bioreactors could be employed to improve oxygen distribution, though these can lead to uneven nutrient distribution because of the uneven concentration gradients (Cai et al., 2023; Kirsch et al., 2023). Bioreactor models such as vertical wheels and wave reactors are used for their low shear impact, preserving cell health under agitation. However, maintaining uniform oxygen levels remains a challenge in large systems where cells may experience hypoxic conditions that affect growth (Martins et al., 2024).

Bioreactors must ensure efficient nutrient delivery while managing metabolite accumulation. In stirred-tank and air-lift bioreactors, cells require a balanced environment to avoid growth-inhibitory metabolite accumulation. For example, ammonia and lactate concentrations can become toxic and inhibit cell growth. Batch and fed-batch systems experience faster accumulation, while perfusion systems allow for continuous refreshment, but it does come at a higher cost (Kirsch et al., 2023). Partial media replacement has been explored

as a strategy to reduce costs by maintaining nutrient balance without frequent complete media changes (Martins et al., 2024). Glucose and amino acid levels must also be carefully managed to avoid excess or deficiency, as these influence cell viability and costs (Martins et al., 2024).

Maintaining bioreactor conditions such as pH, temperature, and oxygen concentration is essential for cell proliferation. Stirred-tank bioreactors are widely used, yet their efficiency relies on precise control over these parameters to avoid cellular stress. Hypoxic conditions have been introduced in bioreactors to increase myoglobin content in cells, which can enhance the appearance and quality of cultivated meat (Chodkowska et al., 2022; Cai et al., 2023). Automated control systems in advanced bioreactors ensure repeatability and are vital for large-scale, commercial operations, though these systems add complexity and potential cost (Gu et al., 2023).

The agitation necessary for nutrient and oxygen distribution in bioreactors introduces shear forces that can affect cell viability. High shear stress can reduce proliferation and lead to increased cell death. Bioreactor designs that minimise shear stress, like low-shear regimes, have shown potential for activating cell-protective mechanisms. Nitric oxide signalling is one pathway that may offer protective effects, though its scalability for large-scale use is still under consideration (Martins et al., 2024; Kirsch et al., 2023).

Commercial-scale cultivated meat production relies on economically viable bioprocesses. Genetic engineering and cell reprogramming have been applied to improve traits like cell proliferation and reduce reliance on external supplements. Modified cells expressing internal growth factors require less frequent media exchange, addressing one of the largest costs in large-scale bioprocesses (Gu et al., 2023). Optimised feeding strategies, such as partial media recycling, have shown effectiveness in managing nutrient and waste levels and reducing costs in industrial setups (Martins et al., 2024).

Finally, achieving consistent product quality is critical to meeting consumer expectations. Addressing these concerns through rigorous testing, clear labelling, and regulatory compliance will be essential to gaining public trust and fostering the adoption of cultivated meat technologies (Gu et al., 2023). All findings are summarised in Table 8 below.

Table 8: Risks and Mitigation Strategies for Bioreactor Design in Cultivated Meat Production.

Category	Identified Risk	Mitigation Strategies
Oxygen Distribution	- Uneven oxygen gradients in stirred-tank and large-scale systems.	- Implement automated control systems for precise oxygen level management.
Nutrient Availability	- Uneven nutrient distribution in large bioreactors. - Toxic metabolite accumulation - Imbalances in glucose and amino acid levels.	- Adopt partial media replacement to reduce costs while maintaining nutrient balance. - Develop optimised feeding strategies to manage key nutrients effectively
Cell Growth Constraints	- Anchorage-dependent growth limiting cell surface area.	- Use microcarriers, cell aggregates, and hydrogel encapsulation to support growth.

	- Potential impact of edible microbeads on taste, texture, and colour.	- Carefully evaluate and test microbead materials to minimise sensory impact on final products.
Environmental Conditions	- Challenges in maintaining pH, temperature, and oxygen concentration. - High shear stress leading - Cellular stress from inconsistent conditions.	- Utilise automated systems for precise control over environmental parameters. - Design bioreactors with low-shear regimes to reduce cellular stress - Explore hypoxic conditions to enhance myoglobin content for better product quality.
Product Quality	- Inconsistent product quality due to variability in cell growth and differentiation. - Consumer distrust in cultivated meat technology.	- Implement rigorous testing, clear labelling, and compliance with regulatory standards. - Focus on transparent processes and product quality to gain public trust.

6.6. Cell differentiation

The next step in the facility involves cell differentiation, where stem cells are guided to mature into muscle fibres and fat cells, or adipocytes, which contain lipid droplets. In animals, the biological mechanisms of cell differentiation are well-established, but replicating these processes in vitro poses unique challenges, the key risks are summarised in Table 9 shown below. Once differentiation is complete, the cells are harvested, and the product can be formed (Martins et al., 2024).

Table 9: Risks and Strategies in Co-Cultivation, Separate Cultivation, and Differentiation for Cultivated Meat production

	Risks	Mitigation strategies
Co-Cultivation	- High variability in gene expression due to interactions between cell types can affect texture and taste consistency. - Co-cultivation requires precise environmental control. Nutrient and oxygen transport become problematic. - Inhibitory crosstalk between cells may suppress essential functions.	- Fine-tune co-culture conditions to minimise crosstalk by controlling specific cell interactions. - Implement perfusion systems to improve nutrient and oxygen transport.
Separate Cultivation	- Difficult to achieve cohesive cell integration. - Complicates tissue heterogeneity due to the need for multiple systems optimised separately.	—
Myogenic Differentiatio	- Replicating muscle fibre development in vitro is challenging due to complex interactions between cell types. - Formation of RCs	- Add specific compounds or ligands to simulate serum starvation effects in serum-free media. - Inhibit signalling pathways to encourage RC fusion into myotubes.

	- Serum starvation methods are imperfect in replicating natural muscle formation, especially under serum-free conditions.	
Adipogenic Differentiation	- Lipid accumulation and metabolic changes are difficult to replicate in vitro. - Species-specific challenges. - 2D culture methods lead to incomplete differentiation.	- Identify small molecules and bioactive compounds. - Optimise media formulations to match the lipid composition of animal fat. - Explore alternatives to insulin
Risks and Safety Concerns	- Additives such as signalling factors, inhibitors, and insulin may not be approved for human consumption. - Antibiotics used to prevent contamination may leave residues. - Residual medium components could pose health risks if not completely removed.	- Use natural plant compounds and fatty acids as differentiation. - Ensure antibiotics are removed or reduced to safe levels. - Thoroughly clear all growth medium components.

6.6.1 Myogenic Differentiation

Myogenic differentiation, the process by which cells develop into muscle fibres, is difficult to replicate outside the body due to the complex interaction of multiple cell types. One in vitro approach, known as serum starvation, induces a temporary pause in cell growth to promote differentiation; however, it still does not fully replicate natural muscle formation, particularly in serum-free conditions. An alternative method involves adding specific compounds to a serum-free medium or using ligands to target cell receptors active in the early stages of differentiation, which simulates the effects of serum starvation.

A key challenge in this process is the formation of reserve cells, which lack essential factors for muscle differentiation, reducing the overall proportion of fully developed muscle cells, which is an issue for cultured meat production. To address this, researchers are exploring the inhibition of certain signalling pathways to encourage RCs to fuse into myotubes, thereby enhancing cellular uniformity and increasing the number of differentiated muscle cells (Martins et al., 2024).

6.6.2 Adipogenic Differentiation

Adipogenesis, the process of fat cell development, is essential for achieving the desired texture and flavour in cell-based meat. It relies on metabolic changes and lipid accumulation, which are challenging to reproduce in vitro. While fat cell differentiation depends on non-serum-dependent signalling molecules, making it somewhat easier than muscle cell differentiation, several challenges persist. Species-specific differences add complexity; for example, bovine lipogenesis relies on acetate rather than insulin, which is commonly used in other species. While insulin can induce fat differentiation in bovine cells, it carries risks of cytotoxicity, altered lipid profiles, and incomplete adipogenesis, resulting in lipid accumulation rather than authentic fat cell development. Additionally, standard 2D culture methods often lead to incomplete differentiation, increasing both time and costs.

To address these issues, researchers are identifying small molecules and bioactive compounds that directly activate adipogenesis regulators, intending to enhance cell maturity and accelerate differentiation. Efforts also focus on optimising media formulations to better match the lipid composition of animal fat, providing cells with ideal substrates for authentic fat development (Martins et al., 2024).

6.6.3 Co-cultivation vs. non-co-cultivation

The production of cultured meat involves two primary approaches to cell cultivation: co-cultivation and separate cultivation, each with distinct advantages, limitations, and risks. Co-cultivation is the simultaneous cultivation of myogenic and adipogenic cells, which can produce structured 3D muscle tissue with improved texture and nutrient composition (Pajcin et al., 2022). Furthermore, co-cultivated cells are capable of secreting factors that promote mutual proliferation and differentiation, enhancing overall cell functionality. However, this approach also presents challenges. Interactions between different cell types can lead to variability in gene expression, potentially compromising the final product's texture and taste consistency (Knežić et al., 2022). The simultaneous cultivation of multiple cell types also demands precise environmental control, which is technically complex. Another significant limitation is the difficulty in nutrient and oxygen distribution across heterogeneous tissues, particularly as the constructs scale up in size. These factors could restrict the scalability of co-cultivation.

Co-cultivation carries additional risks, such as inhibitory cross-talk between cell types, which may suppress essential functions like differentiation. This suppression can introduce variability in gene expression, posing risks to product consistency and quality. Furthermore, the lack of vascularisation in tissue constructs limits the effective transport of nutrients and oxygen, which becomes problematic for larger-scale production (Knežić et al., 2022). Mitigating these challenges requires strategies such as fine-tuning co-culture conditions to control gene expression interactions between cell types, thereby minimising inhibitory cross-talk. The use of perfusion systems could enhance nutrient and oxygen transport, especially when vascularisation is not feasible. Scaffold-based or 3D printing methods may also improve tissue structuring and nutrient distribution, though they introduce risks related to scaffold compatibility and stability.

In contrast, separate cultivation involves growing the cell types independently under tailored conditions. This approach enables precise optimisation of cell-specific environmental factors, which is critical for achieving desired textures and qualities in the final product (Knežić et al., 2022). Separate cultivation utilises bioreactors and specialised media to ensure optimal conditions for each cell type. However, this method also poses challenges. Integrating separately cultivated cells into cohesive tissues can be technically demanding and may require additional steps, such as using scaffolds or other supporting structures, which increases complexity and production costs. The integration process itself can affect the structural and functional properties of the final product, potentially compromising its quality.

Both co-cultivation and separate cultivation present unique advantages and limitations in the context of cultured meat production. Co-cultivation supports mutual cell growth and

differentiation but necessitates careful management of cross-talk and nutrient transport issues. Separate cultivation allows for precise optimisation of cell-specific conditions but introduces challenges in integrating cell types into cohesive tissues. Promising strategies to address these limitations include the use of perfusion systems to improve nutrient transport, as well as advanced scaffold and 3D printing techniques to facilitate tissue structuring and integration (Knežić et al., 2022). These approaches hold significant potential to overcome the inherent challenges of each cultivation method and enable scalable production of high-quality cultured meat.

6.6.4. Other risks and Safety Concerns during cell differentiation

A critical concern in muscle and fat differentiation is the use of signalling factors, inhibitors, insulin, and other additives in the differentiation medium. While effective, these substances are not always approved for human consumption, creating regulatory risks. As a solution, researchers are exploring alternatives, such as natural plant compounds and fatty acids, to promote differentiation without synthetic substances.

Another risk stems from the use of antibiotics in the medium to prevent contamination. While necessary to safeguard the culture, these antibiotics could leave residues in the final product, posing potential risks for consumers, such as allergic reactions or contributing to antibiotic resistance. Ensuring that any antibiotic residues are removed or reduced to safe levels is essential for consumer safety. Similarly, all components used in the growth medium must be fully cleared from the final product to prevent health risks. Researchers are investigating whether residual medium components might have adverse effects on human health if they remain (Cai et al., 2023).

6.7. Cell harvesting and separation

Cell isolation from bioreactors is a critical step in the downstream process CM production, essential for obtaining concentrated cell suspensions required for product formation. After proliferation and differentiation within the bioreactors, cells are separated from the culture medium to remove unwanted components, such as antibiotics, and to recycle valuable nutrients like glucose. The main goal is to collect a cell paste that can either serve as raw material or be assembled into meat-like structures, closely approximating the cell densities found in natural meat tissues. Achieving this requires minimal impact on the cells to preserve their viability and functionality (de Carvalho et al., 2024).

Cell separation poses significant challenges, largely due to the fragility of animal cells. Typical cell separation techniques include centrifugation, tangential flow filtration, dead-end filtration, and sedimentation. Centrifugation is widely used at laboratory scales but poses risks of cell damage under high shear forces, especially in large-scale setups where industrial centrifuges operate at forces upwards of 3000×g. This can cause deformation or lysis of delicate animal cells. Dead-end filtration quickly accumulates a filter cake that increases resistance, limiting its feasibility for large volumes. Tangential flow filtration, which allows continuous filtration, must overcome challenges from high viscosities in cell suspensions that raise the risk of clogging and increased shear stress. Sedimentation is impractical for CM production due to the low density of cells, leading to slow sedimentation rates (de Carvalho et al., 2024).

To address these challenges, several adaptations and strategies are considered to balance effective separation with minimal cell damage, these are summarised in Table 10. In centrifugation, reduced relative centrifugal forces and specialised feed zones can lower the shear forces and avoid excessive cell acceleration. Filtration processes may require continuous removal of filter cake to prevent clogging, while tangential filtration can be optimised by managing suspension viscosities through maintaining lower packed cell volumes. Industrial centrifuges designed for higher robustness, such as disk-stack models, may be operated at lower speeds to adapt them for CM applications. These adjustments aim to retain cell integrity while achieving the necessary separation efficiency, thereby ensuring that only high-quality cell concentrates proceed to the product formation stages in CM production (de Carvalho et al., 2024).

Table 10: Risks and Mitigation Strategies in Cell Separation Processes for Cultivated Meat Production

	Risks	Mitigations
Centrifugatio n	- High shear forces, particularly in large-scale industrial setups, can lead to deformation or lysis.	- Operate industrial centrifuges at reduced speeds. - Employ specialised feed zones. - Utilise disk-stack centrifuges.
Dead-end Filtration	- Quick accumulation of a filter cake. - Elevated viscosity of cell suspensions contributes to	- Implement continuous removal of the filter cake to prevent clogging and maintain process flow.
Tangential Flow Filtration (TFF)	- High viscosities in cell suspensions increase the risk of clogging and shear stress;	- Maintain lower packed cell volumes in suspensions to reduce viscosity. - Design systems with adaptive flow rates to manage shear forces effectively.
Sedimentation	- Low cell densities lead to slow sedimentation rates.	- Not directly mitigable as sedimentation is fundamentally unsuitable for low-density animal cell suspensions. Alternatives such as centrifugation or filtration are preferred.
General Cell Integrity	- High mechanical stresses during separation processes can damage cells.	- Tailor processes to minimise mechanical stresses. - Use equipment designed specifically for handling animal cells
Process Scalability	- Scaling up laboratory methods introduces additional stress on cells due to higher forces and flow rates.	- Tailor large-scale systems to mimic laboratory conditions - Continuously monitor cell integrity during separation to avoid cumulative damage.

7. Interview

The interview focused on the laboratory phase of cultivated meat production, providing insights into key challenges and risks such as contamination, genetic stability, scalability, regulatory hurdles, and consumer acceptance. The discussion spanned the entire production process, from early-stage cell isolation to large-scale production and commercialisation, aiming to outline strategies to address these issues effectively. The insights gained from this interview are summarised in Table 11.

Table 11: Summary of Interview Insights on Challenges, Risks, and Strategies Across the Laboratory Phase of Cultivated Meat Production.

Category	Details	Key Points
Biopsies		
Risks	There are several risks associated with needle biopsies, including cellular damage, contamination, and harm to the animal. Cellular damage reduces the number of viable cells available for cultivation. Contamination risks arise because biopsies are often performed in barns, which are unsterile environments with exposure to microorganisms and debris. Harm to the animal, although minimised, includes potential infections or wounds that heal slowly.	- Cellular damage. - Contamination from unsterile barns. - Potential harm to animals.
Mitigation	To reduce these risks, biopsies should be performed quickly, with samples transported in cold conditions to slow microbial growth. Antibiotics can be used in the collection medium to combat contamination, although this may negatively affect cell viability. Ensuring sterility in the lab and incorporating disinfection steps into workflows further reduces contamination risks.	- Perform quickly and Use cold transport. - Employ antibiotics cautiously and disinfect and sterilise
Comparison	Needle biopsies preferred over tissue biopsies because they are less invasive and better align with animal welfare goals.	- Needle biopsies are preferred.
Challenges	Performing biopsies in barns helps reduce animal stress by keeping them in familiar environments, but this approach significantly increases contamination risks due to the unsterile nature of barns.	- Performing in barns reduces stress but increases contamination risks.
Cell Types		
Primary Cells	Key cell types for cultivated meat include satellite cells (muscle progenitors) and fat progenitors (fibro/adipogenic progenitors or FAPs). Satellite cells differentiate into muscle fibres, forming the texture of meat, while fat progenitors produce the fat necessary for flavour and marbling.	- Muscle progenitors form texture. - FAPs add flavour and marbling.
Insights from Sequencing	Single-cell RNA sequencing is used to identify the diversity of cell types in biopsies, providing insights into the populations present and enabling researchers to select and optimise desired cell types for cultivation.	- Single-cell RNA sequencing identifies cell diversity.
Challenges in Selection	Ensuring the purity of cell populations is a critical challenge. Mixed populations can reduce efficiency, as unwanted cells may interfere with the growth or differentiation of desired cells. Without optimised media formulations or selective isolation methods, it can be	- Mixed populations reduce efficiency.

	difficult to achieve the necessary purity of satellite cells and fat progenitors.	- Purity is difficult without optimised media or methods.
Purification Methods	Selective culture media are formulated to favour the growth of desired cell types while suppressing others. FACS is another key technique, using biological markers to isolate pure populations of satellite and fat progenitors. Proper understanding of cell biology and markers is essential for effective sorting and purification.	- Use selective media. - Employ FACS for precise sorting.
Contamination and Sterility		
Risks	Contamination risks exist throughout the process, from sample collection to lab cultivation. Microbial contamination can occur due to exposure to barn air, open collection tubes, or unclean equipment. Cross-contamination between cell types during isolation is another risk, could compromise the purity of cultures.	- Contamination during collection, lab handling. - Cross-contamination of cell types.
Mitigation	Disinfection steps during cell isolation help reduce contamination risks. Maintaining strict sterility in the lab environment is critical, as is regular monitoring for contaminants using techniques like flow cytometry.	- Use disinfection steps. - Monitor sterility with flow cytometry.
Cell Banking		
Importance	Cell banking is a critical process for scalability and consistency. The master cell bank stores minimally altered cells, serving as a reference for future use, while the working cell bank is derived from the master and used in ongoing experiments or production. This approach ensures a reliable supply of cells and provides a backup for regulatory or experimental requirements.	- Enables scalability. - master cell bank is the reference. - working cell bank supports experiments.
Risks	Freezing cells involves risks such as ice crystal formation, which can rupture cells and reduce viability. Cryopreservation agents like DMSO are toxic to cells if not handled quickly, making the freezing process time sensitive.	- Ice crystal formation can rupture cells. - DMSO toxicity requires fast handling.
Mitigation	These risks are mitigated by working quickly and under cold conditions, using optimised freezing media to balance preservation and toxicity. Ultra-low temperatures (around −150°C) are used for long-term storage to maximise cell viability and stability.	- Work quickly, at cold temperatures and store at ultra-low temperatures - Use optimised freezing media.
Genetic Engineering		
Necessity	Primary cells have limited division potential due to the Hayflick limit, making them unsuitable for long-term cultivation. Genetic engineering is essential to create cell lines that can proliferate indefinitely and improve the efficiency of the production process.	- Genetic engineering overcomes the Hayflick limit. - Enables indefinite proliferation.
Techniques	CRISPR-Cas9 is a widely used tool for genetic engineering, allowing precise modifications to genes that regulate cell growth or behaviour. Gene editing focuses on natural-like changes, such as small deletions or insertions, while genetic modification	- CRISPR-Cas9 for precise editing.

	involves introducing foreign genetic material. Both approaches can improve cell performance, but regulatory and public concerns often surround genetic modification.	- Gene editing vs. genetic modification distinctions.
Risks	Genetic engineering carries risks such as off-target effects, where unintended changes occur in the genome, potentially leading to reduced cell viability, unwanted mutations, or tumour-like behaviour. Regulatory bodies closely scrutinise these processes, particularly in regions with strict laws against genetically modified organisms (GMOs), adding another layer of complexity.	- Off-target effects can cause viability issues. - Strict GMO laws complicate approvals.
Scaling Up to Bioreactors		
Challenges	Scaling up from lab cultures to bioreactors introduces challenges such as shear stress, which damages cells in stirred reactors. Cells in suspension often require microcarriers for attachment, complicating downstream processing. Nutrient and gas gradients in large-scale reactors create uneven growth conditions, further stressing cells and reducing consistency.	- Shear stress damages cells. - Microcarriers complicate processing. - Nutrient gradients affect growth.
Additional Risks	Cells may not adapt well to sudden environmental changes during scaling, resulting in high mortality rates. This stage requires careful optimisation to balance growth conditions and maintain cell viability.	- Environmental shifts cause cell stress. - High mortality risk during scaling.
Broader Risks		
Public Acceptance	Public scepticism about GMOs and unfamiliar technologies is a major hurdle for cultivated meat. In countries like Italy and Romania, cultivated meat has been banned due to concerns about genetic modification. Educating the public and increasing transparency around safety and benefits are essential to improve acceptance and trust.	- Scepticism about GMOs. - Education and transparency are critical for public acceptance.
Financial Viability	The production of cultivated meat remains expensive, particularly due to high costs associated with culture media and bioreactor optimisation. Achieving financial viability is essential to make cultivated meat competitive with traditional meat and accessible to consumers.	- High costs remain a barrier. - Financial viability is essential for competition with traditional meat.
Future Outlook	Overcoming technical challenges, reducing production costs, and addressing consumer concerns are key to the future success of cultivated meat. Advances in genetic engineering and bioprocessing are critical, but public education and clear regulatory frameworks will also play significant roles in enabling widespread adoption and commercialisation.	- Reduce costs and improve education. - Advances in genetic engineering and bioprocessing are essential.

8. Conclusion

This study identified the key risks in the production of cultured meat and explored how a Safe-by-Design approach can mitigate these risks to align with EU safety, ethical, and regulatory standards. Cultured meat offers a transformative opportunity to address global challenges, such as environmental degradation, resource scarcity, and the ethical issues

associated with traditional meat production. However, its success hinges on overcoming significant risks and challenges embedded in a highly interconnected production process and a demanding regulatory environment.

The research identifies that these risks often cluster and interact, meaning that addressing one challenge may unintentionally amplify another. For example, the reliance on FBS simultaneously raises ethical concerns, exacerbates contamination risks, and complicates scalability. Transitioning to serum-free media addresses some of these issues but brings new challenges, such as allergenicity and maintaining consistent cell growth under industrial conditions. Similarly, technical limitations in bioreactors, such as uneven oxygen and nutrient distribution at scale, create feedback loops were scaling compromises cell quality and viability. These challenges are further intensified by biological constraints like genetic drift and the Hayflick limit, which restrict long-term cell proliferation. As such, systemic solutions that address these risks holistically are imperative for the industry to progress.

A Safe-by-Design approach offers a powerful framework for managing these clustered risks. By embedding safety considerations throughout the cultured meat production process, SbD enables producers to anticipate and mitigate challenges proactively. For example, SbD supports the iterative development of serum-free media by focusing on contamination control, allergenicity testing, and optimisation for consistent cell performance. In bioreactor design, SbD emphasises the need for innovations that maintain oxygen and nutrient balance, minimise shear forces, and prevent metabolite accumulation to ensure high cell viability at commercial scales.

The Safe-by-Design approach aligns closely with the EU's precautionary regulatory framework, which emphasises rigorous safety assessments for novel foods. Because of the integration of risk mitigation strategies early in the development process, SbD could simplify regulatory compliance and reduce approval timelines. Additionally, the focus on minimising reliance on animal-derived inputs like FBS addresses ethical concerns, fostering greater consumer acceptance. The implementation of SbD brings significant benefits, but its feasibility varies among stakeholders. Producers face challenges related to costs and the complexity of integrating SbD principles. Regulators, on the other hand, must carefully balance the need for rigorous safety evaluations to foster innovation.

Addressing these challenges requires collaboration among stakeholders. EU policy frameworks need to adapt to better align with the objectives of SbD. Revising the EU Novel Foods regulations to include SbD as a standard could help ensure smoother market access while maintaining high safety standards. Financial incentives could support producers in overcoming cost and complexity barriers, while public awareness campaigns would play an important role in building trust and addressing ethical concerns. Establishing processes to fast-track SbD-compliant products would also simplify market entry and encourage innovation. By focusing on financial support, ethical values, and collaboration, SbD can be successfully integrated into cultured meat production within the EU.

While this study provides valuable insights into the potential of SbD to address key risks in cultured meat production and acceptation, it also highlights notable limitations. Post-separation processes, such as product formation, packaging, storage, and transportation, were not analysed but remain critical to ensuring consumer safety and product scalability. These steps introduce additional risks, including microbial contamination and quality degradation. Furthermore, the sustainability of cultured meat production, particularly its energy, water, and material demand, requires deeper exploration to assess its long-term feasibility. Current gaps in industrial-scale data and the absence of pilot studies leave many findings theoretical, underscoring the need for continued research and validation.

Despite these challenges, the application of SbD principles provides a strong foundation for addressing the interconnected risks in cultured meat production. SbD can enable the proactive resolution of technical, ethical, and regulatory challenges. Future research should focus on later production stages, such as storage and distribution, while also prioritising pilot-scale experiments to bridge the gap between laboratory research and industrial applications.

Cultured meat has the potential to transform the global food system by addressing the environmental and ethical challenges associated with traditional meat production. Realising this potential requires ongoing innovation, collaboration, and adjustments to regulatory frameworks. The SbD approach, by prioritising safety throughout the development process, helps cultured meat meet strict regulatory requirements while also fostering public confidence. With continued dedication, cultured meat can play a key role in creating a more sustainable, ethical, and resilient food system.

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