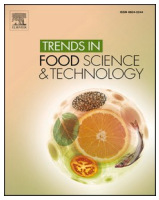
Trends in Food Science & Technology 156 (2025) 104867

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs



Physical stimuli-responsive 3D printable hydrogels for scalable manufacturing of bioengineered meat analogs

Jieun Lee [a,b,1], Sayan Deb Dutta [a,c,1], Tejal V. Patil [a,b], Seong-Jun Cho [d,**], Ki-Taek Lim [a,b,c,*]

[a] Department of Biosystems Engineering, Kangwon National University, Chuncheon, 24341, Republic of Korea
[b] Interdisciplinary Program in Smart Agriculture, Kangwon National University, Chuncheon, 24341, Republic of Korea
[c] Institute of Forest Science, Kangwon National University, Chuncheon, 24341, Republic of Korea
[d] Department of Food Science and Biotechnology, Kangwon National University, Chuncheon, 24341, Republic of Korea

ARTICLE INFO

Handling Editor: Dr AR Jambrak

Keywords:
Cultured meat
3D printing
Biophysical stimuli
Myogenesis
Sustainable management

ABSTRACT

Background: The shift towards sustainable and ethical food systems has accelerated advancements in cultured meat technology. Cultured meat, or lab-grown meat, offers a revolutionary approach to meat production by addressing environmental, ethical, and health issues associated with conventional livestock farming. Traditional meat production contributes to significant greenhouse gas emissio ns, extensive land use, high water consumption, and animal welfare concerns. Cultured meat aims to mitigate these impacts by cultivating muscle tissue *in vitro*, thus reducing the need for animal slaughter and lessening the ecological footprint.

Scope and approach: This review covers cultured meat production, focusing on cell culture fundamentals, including starter cell selection, growth media, and scaffolding. It also examines biophysical stimuli-based platforms for improving muscle cell differentiation and recent advances in 3D printing for customizing tissue structures.

Key findings and conclusion: Challenges remain, such as high production costs and the need for optimized systems and scalable processes. Regulatory and consumer acceptance are crucial for wider adoption. The review highlights progress and obstacles, aiming to support the transition to commercial production and emphasizing the potential of combining physical stimuli with advanced biofabrication to enhance sustainability and reduce costs.

1. Introduction

The global shift towards sustainable and ethical food systems has significantly advanced cultured meat technology. Cultured meat, also known as 'cell-based meat' or 'lab-grown meat' or 'alternative meat', is a real meat produced by growing animal cells in a lab, without raising or slaughtering animals. These products closely mimic the texture, flavor, and nutritional profile of conventional meat while addressing ethical concerns and reducing the environmental impact associated with traditional animal farming. Traditional meat production is linked to significant greenhouse gas emissions, extensive land use, water consumption, and animal welfare concerns (Godfray et al., 2018; Machovina, Feeley, & Ripple, 2015). Whereas, cultured meat represents a revolutionary method for meat production, offering a viable, alternative to traditional methods by cultivating muscle tissue *in vitro*, thereby reducing the need for animal slaughter and the associated ecological footprint (Mancini & Antonioli, 2022; Munteanu et al., 2021). Additionally, it has potential to address critical challenges related to sustainability, ethical issues, growing global protein demand and public health. For example, in 2013 Mark Post and his team cultured first beef burger to demonstrate the advantage of production of meat without extensive land use or livestock farming (M. J. Post, 2014). The process of producing cultured meat involves the cultivation of animal cells in a controlled laboratory environment to form muscle tissue that closely resembles conventional meat in taste, texture, and nutritional profile. This technology hinges on several core principles of cell culture and tissue engineering. The selection of appropriate starter cells, the formulation of optimized growth media, and the development of

* Corresponding author. Department of Biosystems Engineering, Kangwon National University, Chuncheon, 24341, Republic of Korea.
** Corresponding author.
E-mail addresses: sj.cho@kangwon.ac.kr (S.-J. Cho), ktlim@kangwon.ac.kr (K.-T. Lim).
[1] Equally contributed.

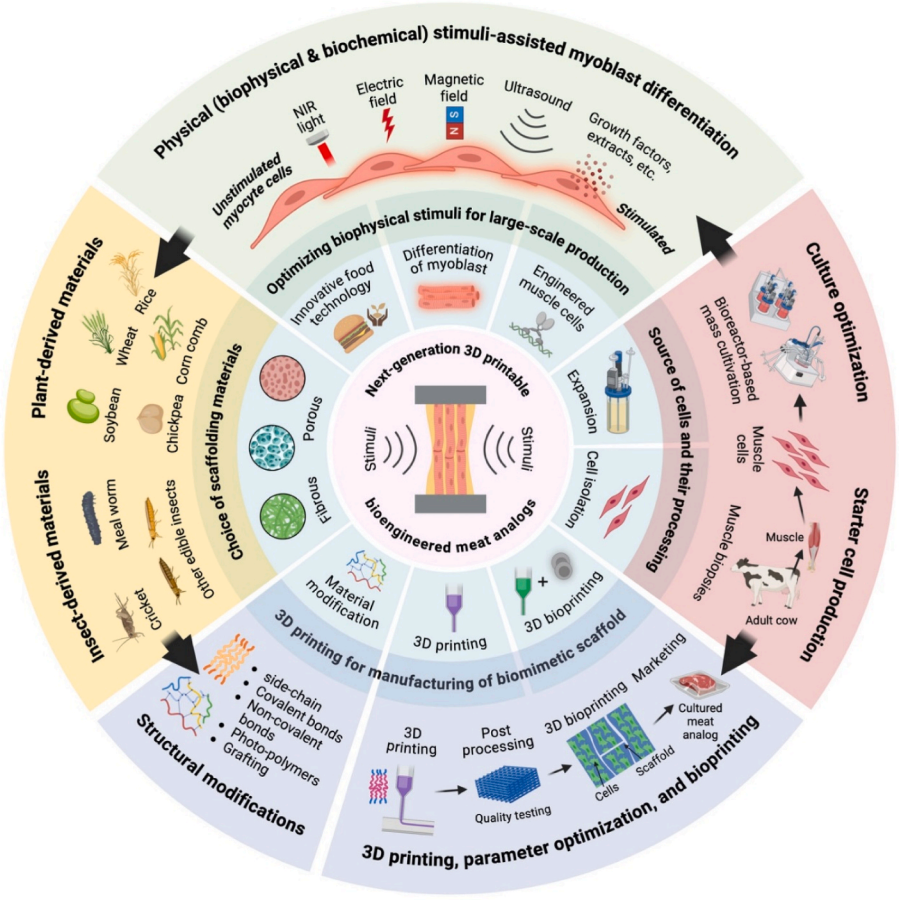
https://doi.org/10.1016/j.tifs.2025.104867
Received 4 September 2024; Received in revised form 31 December 2024; Accepted 3 January 2025
Available online 4 January 2025
0924-2244/

effective scaffolding materials are fundamental to achieving successful tissue development (Chandrababu & Puthumana, 2024; Roy, Panda, & Dey, 2023). Additionally, bioreactor systems are crucial for scaling up the production process and ensuring that large volumes of tissue can be generated efficiently and cost-effectively. Recent innovations in stimuli technologies have further advanced the field of cultured meat. Mechanical and electrical stimulation techniques are employed to enhance muscle cell differentiation and tissue maturation, mimicking the natural physiological conditions that occur in traditional meat. Additionally, bioinspired microfluidic systems are being developed to provide continuous and precise control of nutrient and oxygen delivery, which is crucial for scaling up production and improving tissue quality (Filippi, Buchner, Yasa, Weirich, & Katzschmann, 2022; Johnson et al., 2022).

The application of 3D printing technology represents a significant breakthrough in cultured meat production. 3D printing enables the precise deposition of cells and biomaterials to create complex tissue structures that replicate the intricate architecture of conventional meat. This technology allows for the customization of tissue properties and the production of meat products with specific textures and appearance. Despite the progress made in cultured meat technology, several challenges remain. These include the high cost of production, the need for further optimization of cell culture and bioreactor systems, and the development of scalable manufacturing processes.

Biophysical stimuli play a crucial role in stem cell proliferation and differentiation. Various biophysical stimuli, such as electrical, pressure, fluid flow, magnetic field, and light can induce various transcription factors in muscle cell nucleus and direct to myogenesis (Cedillo-Servin et al., 2024; Mueller et al., 2021). Electrically conductive hydrogels (between 0.5 and 5 V, up to 10 Hz frequency) can effectively trigger the muscle cell alignment and differentiation by activating metabolic pathways and Ca^{2+} influx, resulting in the myosin condensation and inducing the upregulation of sarcomeric actin (SA), myosin G (MyoG), myosin H (MyoH), and myosin heavy chain-IId (MHC-IId) transcription factors (Banan Sadeghian, Ebrahimi, & Salehi, 2018; Khodabukus et al., 2019). Similarly, mechanical force, such as compressive loading/unloading affects the muscle cell alignment and their subsequent differentiation by varying duty cycles, magnitude, frequency, and time. Higher compressive loading (up to 1.2 MPa) would result in less survivability of the myocytes, while a low dose of compression (0.2–1 MPa) would result in higher cell growth and morphogenesis by increasing the intracellular Ca^{2+} influx (Yuan et al., 2024). Similarly, 3D bioprinting, an innovative technology that uses a bioink where muscle cells can be mixed with various printable biopolymers to achieve a 3D biomimetic construct, would also facilitate myogenesis by inducing cell alignment and fusion. By controlling the external pressure and choosing the proper edible biopolymer, we can tune the muscle cell fate by regulating the mechanical and biomechanical microenvironment (H. Lee et al., 2023; Yeo, Lee, & Kim, 2016). Although several advancements have been made in generating stimuli-responsive platforms for myogenesis, the role of biophysical stimuli in the cultivation of cultured meat has yet to be explored. In this regard, various stimuli-responsive hydrogels, nanomaterials, and bioactive components with muscle progenitor cells could be used to study whether these external stimuli conditions can effectively trigger cultured meat production or not.

This review provides a comprehensive overview of the advancements and challenges in cultured meat production, offering insights into the role of various external stimuli-based platforms in regulating myogenesis for cellular agriculture (Scheme 1). It also explains existing and future meat production technologies, from cell sources to the fabrication of advanced biomaterials. By exploring the fundamental principles of



Scheme 1. Schematic illustration of the innovative bioengineering strategies and the role of physical stimuli for the production of meat analogs.

cell culture, scaffold engineering, and the latest biofabrication strategies involving biophysical and biochemical stimuli, this review highlights the progress made and the obstacles that remain. The insights gained will contribute to advancing cultured meat technology and facilitating its transition from the laboratory to commercial production. In particular, 3D printing has emerged as an innovative technology in cultured meat production. It allows for the accurate reproduction of the complex structure of muscle tissue, offering potential solutions to challenges related to texture, mass production, and cost. This review discusses the role of 3D printing and innovative biomaterials in advancing cultured meat technology, focusing on promoting cell maturation and optimizing large-scale production processes.

2. Engineering cultured meat analogs

The demand for tasty and nutritious protein alternatives continues to grow along with consumers' concerns about the environmental impact of mass-producing animal-based foods. In response to this demand, the practice of cultivating or lab-growing animal meat has emerged as a promising approach (Leung, Chong, Fernandez, & Ng, 2023; O'Neill, Cosenza, Baar, & Block, 2021).

Cultured meat-based analogs, also known as lab-grown or cell-based meat, are usually produced by culturing animal cells *in vitro* without the need for animal slaughter (Ismail, Hwang, & Joo, 2020). This process involves extracting stem cells from animals and growing them in a nutrient-rich culture medium within bioreactors, allowing the cells to proliferate and form muscle tissue (Rubio, Xiang, & Kaplan, 2020). These products closely mimic the texture, flavor, and nutritional profile of conventional meat while addressing ethical concerns and reducing the environmental impact associated with traditional animal farming. In contrast, plant-based analogs are made entirely from plant-derived ingredients designed to replicate the taste, texture, and appearance of meat (Boukid, 2021). Common ingredients include soy, pea protein, wheat gluten, and fats like coconut oil, along with natural flavorings. These products are sustainable, eco-friendly, and suitable for vegetarians and vegans, offering popular alternatives such as the Impossible Burger and Beyond Meat. On the other hand, animal-based meat, which is obtained from farmed animals through slaughtering, remains the traditional source of protein, fats, and essential nutrients like iron and vitamin B12 (Y. P. Chen, Feng, Blank, & Liu, 2022). However, it raises ethical concerns related to animal welfare and contributes significantly to greenhouse gas emissions and water usage. While cultured and plant-based analogs represent innovative solutions for sustainable and ethical food production, conventional meat continues to dominate global consumption due to its established production systems and widespread availability. Fig. 1 depicts a survey in cultured meat research over a time period of 10 years (2015–2024). Using a PubMed search tool and keyword '*cultured meat*', it is evident that the number of publications are increasing day-by-day (Fig. 1a). Moreover, when the search algorithm changed to '*3D printing + muscle differentiation*', the number of publications were found higher in last 10 years with a high increase in last 3 years (Fig. 1b). Interestingly, it was also reflected for artificial meat analogs, when a PubMed search was introduced as '*3D printed cultured meat*'. Among the last 3 years (2022–2024), most of 3D printed cultured meat research was conducted mostly with plant-based biomaterials (70–80%, wheat, corn, soybean, and chickpea-based) rather than animal-based (20–30%, edible insect protein-based or alternative growth media-based) biomaterials (Fig. 1c).

Cultured meat can diversify protein production methods, improving the resilience of food systems and ensuring that everyone can access nutritious and protein-rich foods in the future. Cultured meat, also known as cell-based meat or cultivated meat, is a new type of food produced using cell culture, tissue engineering, and food processing technologies (Liu et al., 2023; Yang et al., 2024). Natural meat consists of skeletal muscle, which includes intramuscular fat that contributes to the texture and flavor of meat (David et al., 2023; Purslow, 2018). In

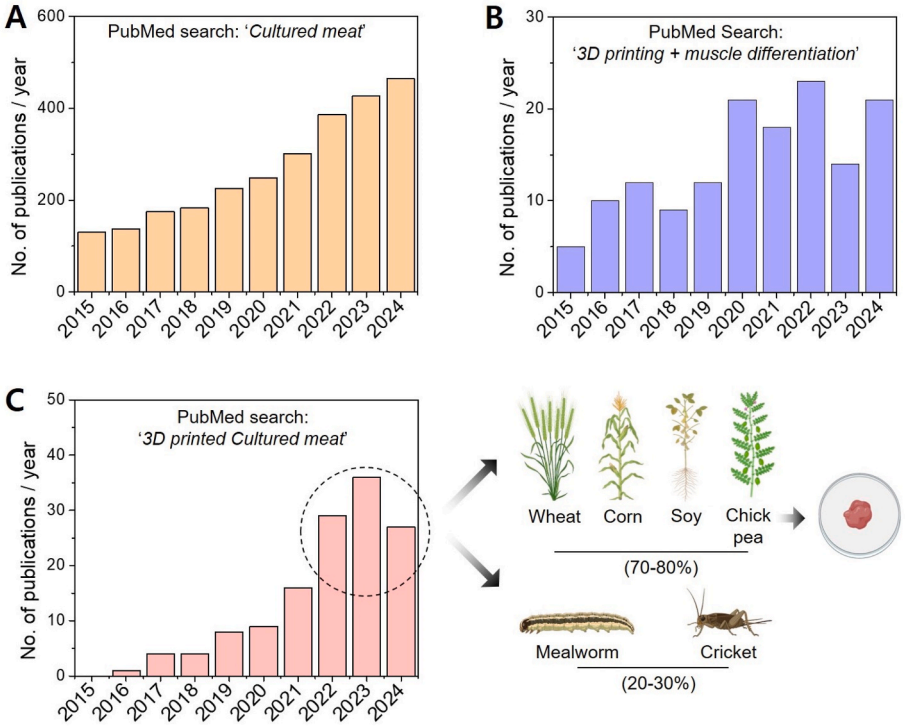


Fig. 1. Year wise bar graphs showing the development in cultured meat production. **(a)** Graph shows the number of publication in each year (2015–2024, 10 years) regarding '*culture meat*' concept, obtained from PubMed search tool. **(b)** Graph shows the number of publication in each year (2015–2024, 10 years) regarding '*3D printing + muscle differentiation*', obtained from PubMed search tool. **(c)** Graph shows the number of publication in each year (2015–2024, 10 years) regarding '*3D printed cultured meat*' concept, obtained from PubMed search tool, of which most of the research focused on plant-derived biomaterials (~80%) than animal biomass-derived (~20%) biomaterials.

addition to muscle fibers and fat tissue, meat also includes other components such as connective tissue and skin (Plikus et al., 2021). The main components of connective tissue and skin are fibroblasts and extracellular matrix (ECM) proteins like collagen, elastin, and fibronectin (K. Sun, Li, & Scherer, 2023). The ECM surrounds and supports muscle fibers and skin layers, forming complex and compact tissue structures that provide strong mechanical strength and various physiological functions to the animal's body (Kumar, Sood, & Han, 2023). Therefore, introducing fibroblasts into cultured meat production to increase ECM content can enhance structural integrity and improve the nutrition and texture of cultured meat (Alam et al., 2024). Creating skeletal muscle with fat requires various cell types, which have different scaffold requirements that are challenging to achieve in co-culture. Generating edible scaffolds with physical properties tailored to different cell types, including muscle and fat cells, can provide a solution for engineering multi-component cultured meat with improved texture, tenderness, juiciness, and flavor (Kawecki et al., 2023). However, challenges still need to be addressed regarding the nutrition, structural characteristics, and taste of cultured meat. One of the major barriers to scaling up cultured meat production is the technical challenge of engineering complex tissues composed of multiple cell types and components *ex vivo* (M. J. Post et al., 2020). This issue is amplified by the demands for mass production, low cost, and, most importantly, commercial viability. In particular, 3D printing technology has emerged as a crucial innovative tool in cultured meat production. By utilizing 3D printing, the complex structure of muscle tissue can be accurately reproduced, offering the potential to solve issues related to texture, mass production, and cost reduction (Portanguen, Tournayre, Sicard, Astruc, & Mirade, 2019). This plays a key role in implementing various cell types and complex tissue structures, contributing to the improvement of cultured meat quality. Scaffold engineering using 3D printing provides an environment where different cell types can bond effectively, creating scaffolds with physical properties tailored to the needs of each cell type, thus playing a significant role in enhancing the texture, tenderness, juiciness, and flavor of cultured meat (Rao, Choi, & Han, 2023). Furthermore, 3D printing helps ensure the efficiency required for large-scale production, bringing us closer to commercial production.

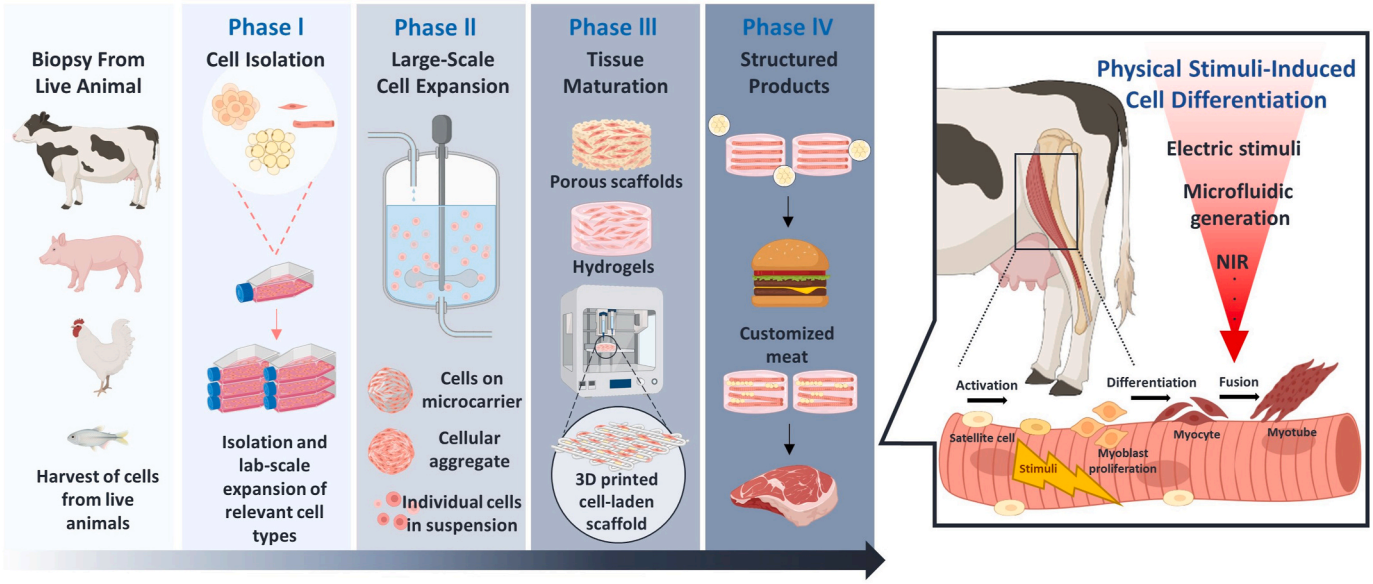
2.1. *Fundamental principles of cell-based meat production*

In this section, we discussed the fundamental aspects of cell-based meat production in detail, including cell sources, scaffolding materials, and large-scale production processes.

Cell culture production for artificial meat can be broadly categorized into four stages. For culture meat cells, these stages are (1) isolation and acquirement of cells, (2) proliferation and growth of cells, (3) promotion of cell differentiation and maturation on a scaffold, and (4) processing into a food product (Xiang et al., 2022). Scheme 2 provides a general overview of the typical production process, illustrating the various stages of producing artificial meat.

2.1.1. *Starter cell selection (phase 0)*

The selection of cell sources poses a significant challenge in cultured meat production. The key issue lies in obtaining a sufficiently large number of uniform starter cells for effective proliferation and differentiation. Over the past decades, various stem cell types have been identified, and the related technology has advanced considerably (Asakura, Rudnicki, & Komaki, 2001; Asakura, Seale, Girgis-Gabardo, & Rudnicki, 2002; Peng & Huard, 2004; Van Eelen, Van Kooten, & Westerhof, 1999). For example, Mark Post and coworkers used bovine skeletal muscle satellite cells to produce hamburger (M. J. Post, 2014). Kadim and group also showed the development of cultured meatballs by cultivation of bovine and avian muscle cells whereas, Lew et al. showed that adipocytes (fat cells) were cultured to enhance the flavor and texture of cultured beef meat (Kadim, Mahgoub, Baqir, Faye, & Purchas, 2015; Lew et al., 2024). Currently, several cell sources are applied to tissue engineering. One source involves original tissues or cell lines. Mutations are induced through genetic engineering or chemical methods, resulting in unlimited cell proliferation, thereby reducing dependence on fresh tissue samples and increasing the rate of cell proliferation and differentiation (Zidarič, Milojević, Vajda, Vihar, & Maver, 2020). However, there are challenges associated with cell lines derived from stem cells, including genetic instability, phenotypic drift, and issues like misidentification and contamination with microorganisms (Tuomisto & Teixeira de Mattos, 2011). Few aspects can be considered to overcome these challenges. For example, genetic stability can be ensured through regular genomic screening, using early passage cells, maintaining optimized growth conditions, and minimizing selective pressure during cell expansion (Poetsch, Strano, & Guan, 2022). To mitigate phenotypic drift, standardized differentiation protocols, chemically defined culture media, and the use of cryopreserved cell banks help maintain consistency. Preventing cell misidentification involves routine DNA



Scheme 2. Overview of the cultured meat production process: Integrating scaffolds and physical stimuli for enhanced tissue development.

authentication methods like Short Tandem Repeat (STR) profiling, strict documentation, and quality control measures (Reid, Storts, Riss, & Minor, 2013). Similarly, microbial contamination can be minimized by employing aseptic techniques, closed-system bioreactors, antibiotic-free cultures, and regular testing for contaminants (Sogore et al., 2024). Additionally, integrating advanced technologies such as CRISPR-Cas9 for gene correction, artificial intelligence for real-time monitoring, and automation for precise handling can further enhance cell line reliability and safety (Galanakis, 2024). These approaches are essential for producing consistent, high-quality cultured meat at scale.

Another source of tissue engineering for cultured meat includes stem cells isolated from tissues, such as embryonic stem cells, muscle stem cells, and mesenchymal stem cells. Muscle stem cells, or satellite cells, are widely used in cultured meat research due to their differentiation potential (Hill, Bressan, Murphy, & Garcia, 2019). Embryonic stem cells are considered an ideal choice for cultured meat production due to their potential for unlimited proliferation and the ability to differentiate into all cell types required for meat production (Kim et al., 2024). However, developing embryonic stem cell lines from animals poses challenges, facing cell contamination and reduced replicating efficiency. Other cell types, including adult stem cells, are considered an alternative. Stem cells derived from mature tissues, such as adult stem cells from muscle or adipose tissue, are gaining attention as a viable resource for meat production. Cells are purified using specific surface markers to ensure healthy cultivation and differentiation. During proliferation, these cells can differentiate into specific types through chemical, biological, or mechanical stimulation (Arshad et al., 2017). Although stem cell lines theoretically have unlimited growth potential once established, the accumulation of mutations during proliferation often impacts their amplification ability, leading to eventual cell aging.

2.1.2. Choice of cell and growth components (phase I)

Efficient, safe, and large-scale cultured meat production depends on selecting appropriate cell sources. Stem cells, including those derived from tissues or transformed into induced pluripotent stem cells (iPSCs), offer potential solutions. Strategies to enhance cell proliferation involve addressing the Hayflick limit through telomerase regulation. Patterned co-culture techniques and consideration of co-culturing with other cells, such as adipocytes, are explored to improve the quality of cultured meat (Choi & Myung, 2014; Hossner, Yemm, Vierck, & Dodson, 1997; X. Sun & Zemel, 2009).

A pivotal aspect of cultured meat production involves the formulation of a nutrient-rich growth medium. Traditionally, growth factors and serum have been essential components, often sourced from fetal bovine serum (FBS). However, ethical and safety concerns have fueled research into serum-free media formulations (Bjare, 1992; Tan et al., 2015). The use of antibiotics in growth media, which is standard in traditional cell culture, raises controversy in cultured meat production due to potential antibiotic resistance issues. Ongoing research explores animal-free and antibiotic-free alternatives to enhance ethical and sustainable practices (Kolkmann, Post, Rutjens, Van Essen, & Moutsatsou, 2020). For example, Messmer and colleagues conducted a study to examine the differentiation of bovine satellite cells into mature muscle fibers without the utilization of animal-derived components (Messmer et al., 2022). The results of this investigation provide support for the advancement of a serum-free methodology for the production of cultured beef, which is appropriate for the fabrication of three-dimensional bioartificial muscle constructs.

2.1.3. Bioreactor systems for large-scale expansion (phase II)

Bioreactors are essential for scaling up cultured meat production by providing a controlled environment for cell growth and tissue development. This process involves cell line development, growth media formulation, scaffold design, and bioreactor implementation to ensure high-density cell culture, nutrient distribution, and waste removal under optimal conditions. Bioreactors enable precise control of pH, temperature, and shear forces, supporting scalable tissue engineering and maintaining consistent product quality (Lim et al., 2022). Advanced systems incorporate microfluidic and perfusion technologies to improve nutrient distribution and prevent necrotic zones in dense tissues, enhancing scalability. However, large-scale implementation faces challenges such as high costs, compatibility with 3D scaffolds, and maintaining gas exchange and nutrient delivery in thick tissues. Traditional designs, optimized for microbial cultures, often fail to meet the demands of mammalian cells, while shear stress from mechanical agitation may damage fragile cells, reducing yields (Martin & Vermette, 2005). To address these issues, scaffold-integrated bioreactors, including hollow-fiber systems and microcarrier-based designs, improve scalability and nutrient delivery (Jankovic et al., 2023). Low-shear stirring mechanisms and dynamic perfusion systems help reduce mechanical stress and enhance oxygenation. Cost reduction strategies, such as synthetic growth media and plant-based supplements, lower dependency on expensive growth factors, while AI-driven monitoring systems optimize process conditions in real time (Nikkhah et al., 2023; Quek et al., 2024). Incremental scaling with modular designs further supports flexible production. Research also focuses on integrating renewable energy sources and process automation to reduce energy consumption and costs (Kasani, Esmaeili, & Golzary, 2022). Establishing regulatory frameworks and standardized protocols ensures compliance, safety, and market readiness, paving the way for cultured meat to become a viable and sustainable alternative to conventional meat production.

2.1.4. Scaffolding materials for cell maturation (phase III)

Scaffolds are crucial in providing a framework for adherent cells, such as myosatellite cells, in cultured meat development. Key properties of effective scaffolds include biological activity, large surface area, flexibility, and optimal porosity for growth medium diffusion (Chung & King, 2011). When surface-modified, materials like cellulose, alginate, chitosan, or collagen facilitate the mechanical stretching of myoblasts. Gelatin-based scaffolds, for instance, have been utilized to create hydrogels with enhanced flexibility and biocompatibility, mimicking natural ECM properties for improved cell adhesion and proliferation (Ben-Arye et al., 2020). Similarly, soy protein-based scaffolds offer an economical and plant-derived alternative with high mechanical strength, making them suitable for 3D bioprinting applications in cultured meat production (Wei et al., 2023). The emergence of 3D bioprinting technology allows for precise control over cell positioning and densities, ensuring cultured meat without compromising texture. However, several limitations hinder its practical application. For example, printing speed remains a challenge, as current bioprinting technologies can be slow, making large-scale production inefficient (Guo, Wang, He, Hu, & Jiang, 2024). Additionally, the cell survival rate can be compromised due to shear stress during the printing process or suboptimal post-printing conditions (Barbosa et al., 2023). Cost-effectiveness is another concern, as bioprinting materials, equipment, and maintenance are expensive, limiting scalability for mass production. Finally, the risk of contamination during the printing process, especially in open systems, poses challenges for maintaining sterility and product safety. To overcome these challenges several strategies can be implemented. For instance, to improve printing speed, bioprinter with multiple nozzles can be used to simultaneously print different cell types and materials, and advanced techniques such as laser-assisted bioprinting or continuous extrusion-based printing can accelerate the process (Ravanbakhsh et al., 2021). To enhance cell survival rates, low-shear stress extrusion methods could be used, optimized biocompatible hydrogels can be employed for better cell protection, and ensuring optimal post-printing conditions like temperature and nutrient supply. Additionally, to make the process more cost-effective, the development of low-cost bioinks, integration of automation and robotics, and scaling production to reduce per-unit costs are crucial. Similarly, preventing contamination can be achieved through closed-loop bioprinting systems, strict aseptic techniques, and real-time contamination monitoring. Additionally, adopting

artificial intelligence and machine learning for optimizing print parameters, incorporating in-line quality control measures, and using bioreactors for tissue maturation can further support the consistency and safety of the final product. These advancements are key to making 3D bioprinting a more efficient and scalable technology for cultured meat production.

2.1.5. *Quality assurance, production, and commercialization (phase IV)*

This is the final stage of cultured meat production, which includes quality assurance (=biosafety), ethical approval, political and regulatory aspects, followed by successful commercialization (Bhat, Morton, Mason, Bekhit, & Bhat, 2019). After the *in vitro* culture of the meat analog, it should be tested in terms of color, texture, and taste in order to convince a consumer (Baig et al., 2024). The consumer acceptance of cultured meat is one of the important steps of commercialization, which also reflects the market value (Hanan, Karim, Aziz, Ishak, & Sumarjan, 2024). So far, the acceptance of cultured meat or lab-grown meat is still indigestible to consumers owing to the lack of knowledge, and fewer restaurants are accessible for dining. Looking forward, the awareness of cell-based meat over conventional meat is crucial for the successful commercialization of cell-based meat towards sustainable agriculture.

2.2. *Innovative biomaterials as a scaffold for cultured meat*

When designing scaffolds for cultured meat, important considerations include cell support, technical feasibility, safety, sustainability,

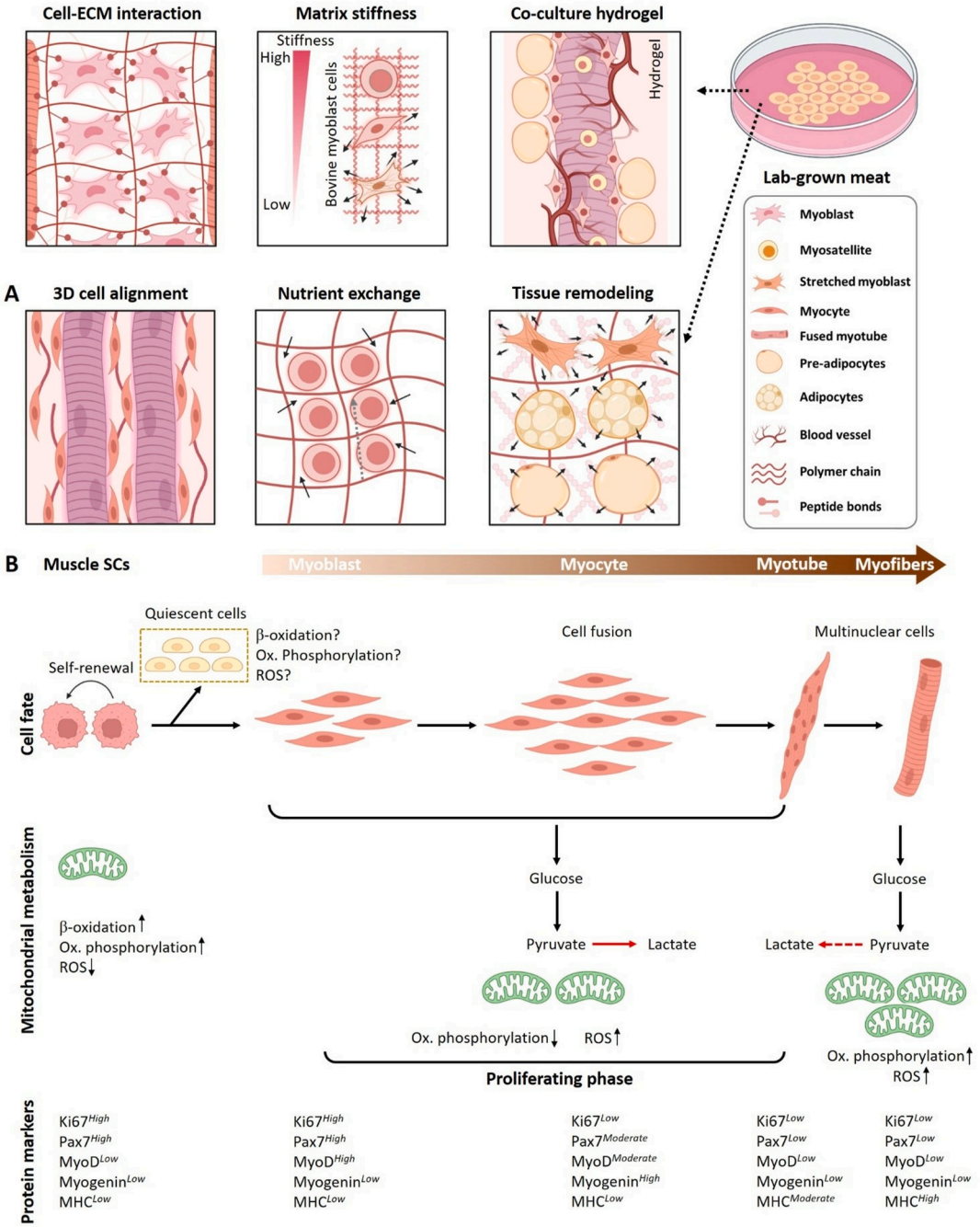


Fig. 2. **(A)** Considerations for biomaterial properties in optimal tissue engineering of cultured meat. **(B)** Overview of myogenic differentiation. Activated satellite cells (SCs) either differentiate into myoblasts or adopt a quiescent reserve cell phenotype to maintain the muscle stem cell pool.

and commercial viability, as the chosen biomaterials can impact all product aspects. Biomaterials used for cell cultivation should provide specific mechanical and biochemical cues that guide cell attachment, morphology, proliferation, and other cellular activities (Fig. 2A) (Cai et al., 2020). Furthermore, the selected biomaterials can provide microenvironments that support or direct the differentiation of meat-relevant cell types such as muscle cells, fat cells, or fibroblasts (Fig. 2B). Polysaccharides used as scaffolds for cultured meat support cell adhesion and proliferation. For example, chitosan, which is edible, cost-effective, and derived from marine biowaste, offers good cytocompatibility and sustainability. However, its acceptance may be limited due to its animal-derived origin. In contrast, plant-derived polysaccharides such as alginate, agarose, pectin, pullulan, gellan gum, hemicellulose, and starch are widely accepted (Heidarian et al., 2020; Levi, Yen, Baruch, & Machluf, 2022). Approved by the FDA as food additives, these polysaccharides are used in various food applications and offer functional cell-binding domains, making them valuable alternatives (Jin, Liu, & Jiang, 2021). Table 1 provides a comprehensive summary of the raw materials, types, target cells, and applications of scaffolds used in cultured meat production. This table offers valuable insights for evaluating the suitability of raw materials and cell types during scaffold design. Additionally, it allows for a clear understanding of the potential applications and related research trends, making it a critical reference for cultured meat development.

2.3. Key considerations in the production process

2.3.1. Serum-free culture for cost reduction

The cultivation of bovine satellite cells (BSC), related to meat production, has traditionally relied on components such as fetal bovine serum (FBS) (M. J. Post et al., 2020). However, this cell cultivation method has received negative evaluations due to low sustainability and inadequate consistency. Moreover, FBS, being of animal origin, may conflict with the goals of meat production (Stout, Mirliani, Soule-Albridge, Cohen, & Kaplan, 2020). Recently, there have been attempts to develop serum-free media for expanding bovine satellite cells. For example, a study performed by Amirvaresi et al. highlights the potential of alfalfa protein isolate as a sustainable alternative to FBS for the proliferation of bovine satellite cells in cultivated meat production (Amirvaresi & Ovissipour, 2024). It found that a lower concentration of alfalfa protein significantly enhanced cell growth. However, the research emphasizes the need for further exploration of various protein sources to optimize cell culture media. However, these serum-free media formulations have encountered challenges such as lower performance than traditional serum-containing media, dependency on exclusive or animal-derived additives, and concerns about regulatory issues such as synthetic steroids (Humbird, 2021). Additionally, there has been insufficient verification of the serum-free media's effectiveness for the continuous expansion of satellite cells. For example, recent studies have tested commercially available serum-free media and supplements, revealing the limitations of using serum-free media (Kolkmann et al., 2020). While serum-free media such as FBM™ and Essential8™ did not reach the high benchmark levels of cell proliferation seen with 20% FBS and 10% horse serum (HS), they did continuously support bovine satellite cell proliferation. On the other hand, Lipogro™ induced a phenotypic change, causing satellite cells to resemble adipocytes, which indicates that such formulations are unsuitable for cultured meat production. The research also explored partial medium replacement, where 25% of the medium's components were replaced with cell-derived products, resulting in increased growth in some serum-free media. These findings highlight the potential for serum-free media to support cell growth but also suggest the need for further optimization. Despite its potential, serum-free media still face significant challenges regarding effectiveness, cost-efficiency, and scalability. Further research is needed to develop animal-free, sustainable serum alternatives.

In conclusion, the use of serum-free media in cultured meat production remains an area that requires ongoing research and improvement, with the development of serum-free media that are safe and completely free of animal-derived components being necessary (S. Y. Lee et al., 2022).

2.3.2. Bioreactors and scaling challenges

Bioreactors play a pivotal role in cultured meat production, serving as controlled environments for cell proliferation and tissue development (W. Sun et al., 2024). They provide the necessary conditions for cell growth, including nutrient supply, oxygenation, and waste removal, thereby mimicking the natural physiological environment required for muscle tissue formation. The scalability of bioreactors is fundamental to transitioning cultured meat production from laboratory-scale research to commercial manufacturing (Manzoki et al., 2024). However, significant challenges must be addressed to achieve this transition effectively.

Bioreactors are essential for enabling high-density cell culture and maintaining consistent growth conditions (de Mello et al., 2024). They allow precise control over parameters such as pH, temperature, oxygen levels, and agitation, which are critical for cell viability and differentiation. Furthermore, bioreactors facilitate the homogeneous distribution of nutrients and gases, ensuring uniform tissue growth. Their ability to support scalable tissue engineering processes makes them indispensable for large-scale production, positioning cultured meat as a sustainable and ethical alternative to traditional meat. Despite their importance, several challenges hinder the scalability of bioreactors. Traditional bioreactors are primarily designed for microbial or suspension cell cultures, which differ significantly from the requirements of adherent mammalian cells used in cultured meat production (Zheng, Hu, & Zhou, 2024). Cells require 3D scaffolds and attachment surfaces, necessitating specialized reactor designs with integrated scaffold support systems. Ensuring uniform distribution of nutrients and oxygen in large bioreactors is another challenge, particularly for dense and multilayered

Table 1
Summary of the types of scaffolds used in cultured meat production and their intended purposes.

Scaffolds	Raw material (s)	Scaffold type(s)	Cell type(s)	Applications	Ref.
Animal-derived materials	Gelatin	Hydrogel	C2C12	Non-mammalian based for *in vitro* meat production downstream	Enrione et al. (2017)
	Gelatin	Micropatterned Film	C2C12	Culture of *in vitro* meat based on a non-mammalian scaffold	(Acevedo et al., 2018; Orellana et al., 2020)
	Chitin	Synthetic	Unspecified	Patent on the industrial production of *in vitro* meat with potential scaffolds mentioned	(Van Eelen et al.,1999)
	Collagen	Edible Non-edible (removed in the production process)			
	Collagen	Mesh sheet/film	Unspecified	A review of the possibility of producing *in vitro* meat via tissue engineering techniques and scaffold suggestions	Edelman, McFarland, Mironov, and Matheny (2005)
Plant-derived materials	Textured soy protein	Textured soy protein	Bovine satellite cells	Demonstrates the possibility of textured soy protein as a scaffold to support bovine satellite cell attachment and proliferation in creating a 3D muscle tissue	Ben-Arye et al. (2020)

tissues, where inadequate diffusion may lead to necrotic zones and reduced product quality.

Mechanical agitation and fluid flow in bioreactors can introduce shear forces that damage fragile mammalian cells, leading to lower yields and compromised tissue integrity (Palladino et al., 2024). Moreover, current bioreactor systems are expensive to build and operate, posing a major barrier to cost-competitive production (Akram, 2024). Energy-intensive processes further increase operational costs, making economic feasibility a pressing concern. Scaling up also requires advanced real-time monitoring systems to track cell growth, metabolic activity, and environmental parameters. Existing systems often lack the precision and adaptability required for cultured meat applications. To address these challenges, several strategies have been proposed. Developing scaffold-integrated bioreactors that support adherent cell growth while enabling efficient nutrient and gas exchange is critical. Designs such as hollow-fiber bioreactors and perfusion-based systems can address transport limitations and improve scalability (Thangadurai, Srinivasan, Sekar, Sethuraman, & Sundaramurthi, 2024). Incorporating low-shear stirring mechanisms, microcarrier, and advanced microfluidic systems can reduce shear stress and enhance nutrient distribution, ensuring higher cell survival rates (Gome et al., 2024). Implementing sensors and AI-driven systems for real-time monitoring and feedback control can optimize environmental conditions, reducing variability and improving reproducibility (Todhunter et al., 2024). Reducing dependency on expensive growth factors by engineering synthetic growth media and leveraging plant-based formulations can significantly cut costs while maintaining cell proliferation efficiency. Incorporating renewable energy sources and process optimization algorithms can reduce energy consumption and lower operational costs. Developing modular bioreactor systems that can be scaled incrementally allows manufacturers to test and optimize processes before transitioning to full-scale production (Pasitka et al., 2024). Ongoing research is focused on integrating bioreactors with advanced tissue engineering approaches to replicate complex meat structures (Fasciano, Wheba, Ddamulira, & Wang, 2024). Efforts are also being made to standardize production protocols to meet regulatory approvals and ensure consumer safety. With continuous advancements in bioreactor technology, coupled with interdisciplinary collaborations, scaling up cultured meat production can become economically viable, environmentally sustainable, and widely accepted.

In conclusion, the intricate process of cultured meat production involves selecting appropriate cells, optimizing growth media, developing suitable scaffolds, and addressing challenges in bioreactor implementation. Ongoing research and technological advancements aim to overcome these challenges and pave the way for a sustainable and ethical future in cultured meat production.

2.3.3. Stimuli-assisted technologies

Research related to skeletal muscle cell cultivation well demonstrates the importance of stimulation techniques for effective cultured meat production. Most mesenchymal cells form rigid fibers that generate tension, creating structures of collagen or collagen/matrix gel (Sarrigiannidis et al., 2021). These structures play a crucial role in dramatically increasing protein production in bioengineered muscle (Pentidis, 2024). However, applying cyclic stretching has sometimes shown insufficient improvement or even negative effects on protein synthesis, which conflicts with other studies observing positive effects on muscle maturation (Boonen et al., 2010; Mueller et al., 2021). This discrepancy highlights the need for further research. In addition to passive stretching and tension, studies are exploring electrical stimulation to stimulate protein and force production. Applying electrical stimulation with specific coatings can lead to the early maturation of skeletal muscle fibers. Current discussions on mechanical stimulation for cultured meat suggest that it is possible to generate small-scale bioengineered muscle (BAM) with limited nutrient and oxygen supply using existing technologies (Skardal, Zhang, & Prestwich, 2010). However, attempts to create large BAMs with internal vascular or channel systems have not yet been made. The development of printing and biomaterial technologies indicates potential future advancements. Moreover, proteins other than contractile proteins, such as myoglobin, play significant roles in the texture, color, and flavor of cultured meat (Y. P. Chen et al., 2022; Sha & Xiong, 2020). Myoglobin, responsible for the pink color of meat and iron transport, is regulated by transcriptional activators such as MEF2, NFAT/calcineurin, and PGC-1α (M. Post & Van Der Weele, 2020). Hypoxic conditions maximize myoglobin stimulation. Therefore, using compatible stimulation techniques to increase myoglobin content in cultured meat is crucial. While current technologies enable skeletal muscle cultivation, optimizing stimulation techniques, such as electrical and mechanical stimulation, is crucial for improving protein synthesis and enhancing the texture and flavor of cultured meat. This optimization plays a key role in bridging the gap between laboratory-scale production and large-scale commercial viability.

In conclusion, improving stimulation techniques is essential for enhancing protein production and quality. By focusing on passive stretching, electrical stimulation, and adequate nutrient and oxygen supply, the commercialization potential of cultured meat can be significantly increased.

3. Biofabrication strategies for cultured meat production

In recent years, the technology known as 3D printing or additive manufacturing (AM) has gained significant attention as a tool for mass-producing complex geometric structures using various materials such as metals, ceramics, and polymers (Sinke, 2021). 3D printing technology is a process that uses computer-aided design (CAD) and computer-aided manufacturing (CAM) software to create freeform structures (Levi et al., 2022). The created design is then converted into an STL file using slicing software, and a three-dimensional object is formed by depositing multiple layers (Dick, Bhandari, & Prakash, 2019). This technology has emerged as a promising tool for cultured meat production due to its versatility, precision, and reproducibility. By combining various food materials with 3D printing methods, it has become possible to develop new processes for food customization, including all acceptable requirements such as unique textures, nutritional value, and flavors (Ding et al., 2023; Schüler et al., 2024). As a result, several companies are currently developing 3D printing-based platforms for cultured meat production, and some have already realized the commercialization potential of this approach. Examples of such companies include American BlueNalu (fish, seafood), MeaTech from Israel (beef, poultry), Aleph Farms (beef), and Future Meat (various species) (Elemoso, Shalunov, Balakhovsky, Ostrovskiy, & Khesuani, 2020). MeaTech and Aleph Farms are focused on developing products that mimic the texture of natural whole meats, BlueNalu plans to print thin fish fillets, and Future Meat is emphasizing the development of bioreactor systems. However, to manufacture printed food with the desired design and nutritional value, several aspects must be considered to ensure the necessary printing precision and accuracy.

3.1. 3D printing/bioprinting for cellular agriculture

3D printing of complex structures such as muscle, skin, bone, and cartilage using bio-materials is referred to as bio-printing. Bio-printing is a new technology based on tissue engineering, and this field is still under development for food applications. The 3D printing of meat mainly consists of initial cells, culture media, and scaffolds. Initially, cells need to have the ability to self-renew, proliferate indefinitely, and differentiate to develop and form the necessary cells for meat (*e.g.*, muscle cells, fat cells, and cartilage cells). The media primarily drives cell growth, proliferation, and differentiation by providing the nutrients necessary for cell growth, leading to tissue regeneration and maturation. Growth factors and animal serum are crucial components of the growth media for tissue maturation and are often derived from animal embryos. 3D

scaffolds are essential for the structure of cultured meat, determining its physiological similarity to real meat. Therefore, the scaffolds used in 3D printing must have excellent sensory quality and food safety, ensuring sufficient surface area and porosity to support cell adhesion and proliferation. To ensure the safety of 3D scaffolds for cultured meat, it is essential to use biocompatible, food-grade materials like collagen and gelatin, which comply with food safety regulations (Bomkamp et al., 2022). Contamination during printing can be minimized using closed-loop systems and aseptic techniques, along with sterilization methods such as UV exposure or gamma radiation (Shyam & Palaniappan, 2023). Toxicity and residue testing should be conducted to detect harmful substances, while mechanical testing ensures structural integrity. Regular microbial monitoring helps maintain safety, and post-printing biocompatibility and sensory evaluations ensure the final product is safe for consumption. Adherence to regulatory standards and a traceability system ensure transparency and safety in the production process (Bomkamp et al., 2022).

3.1.1. 3D printing of cell-cultured meat

Two methods are generally used for 3D bio-printing cell culture. One involves inoculating printed scaffolds with cells and then culturing them, while the other involves printing scaffolds using hydrogels that contain cells. For instance, steak-shaped cultured meat was successfully manufactured by combining cells with hydrogel via a digital light processing (DLP) printer (Fig. 3A) (Jeong et al., 2022). To enable concurrent cultivation of muscle and fat, they added oleic acid to create a

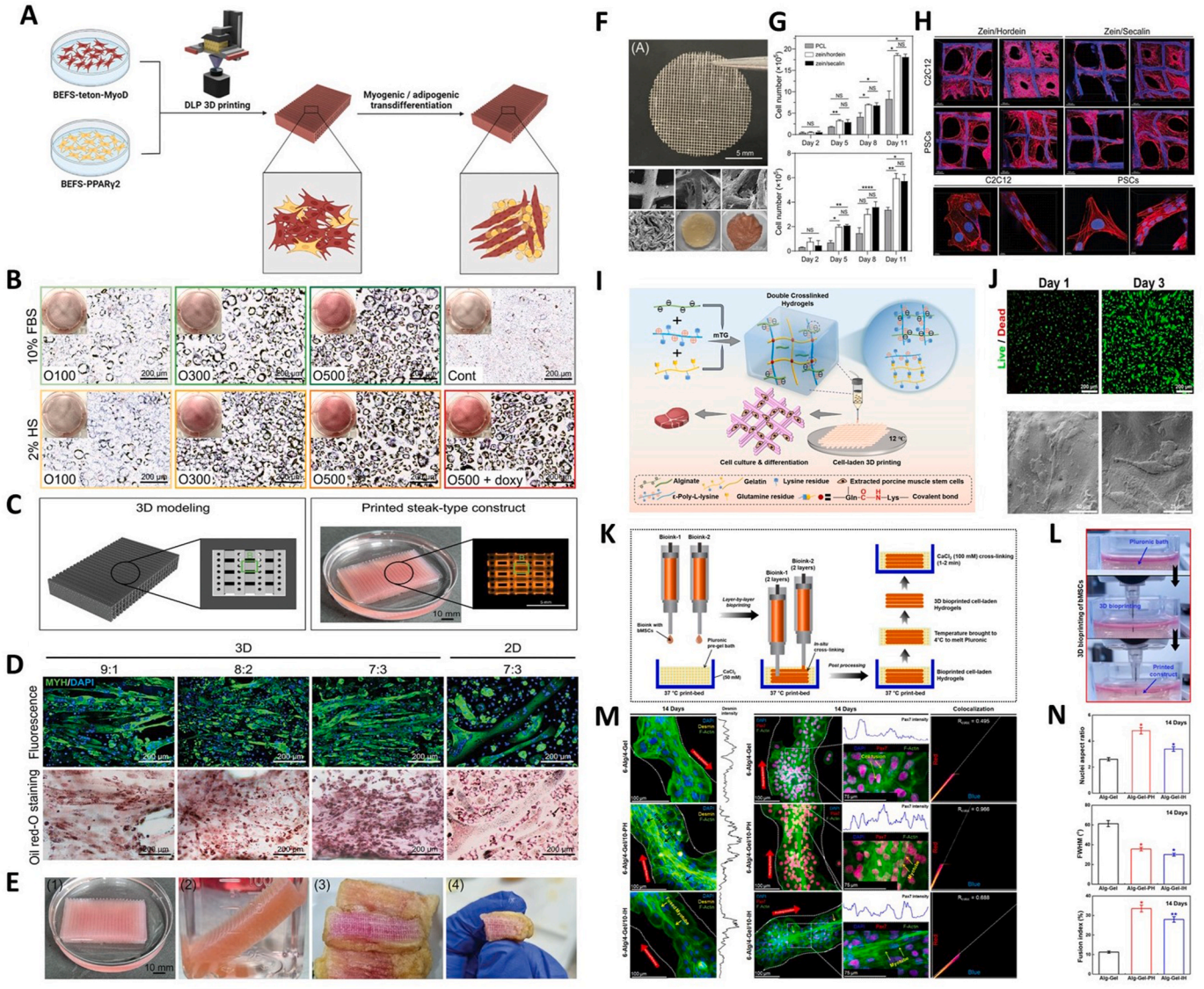


Fig. 3. **(A)** Schematic diagram of the 3D-bioprinted steak-type cultured meat production process. **(B)** Oil Red O staining of BEFS-PPARγ2 cells in various conditions. Scale bar = 200 μm. **(C)** 3D design (left) of the steak-type cultured meat and a photograph of printed steak-type cultured meat (right). **(D)** Immunofluorescence image of simultaneous myogenesis and adipogenesis. **(E)** Photographs of steak-type cultured meat (1, 2) and the cross-section (3, 4) (Jeong et al., 2022). **(F)** Photograph of a circular zein/decalin scaffold and SEM images of the scaffold after seeding with C2C12 cells. **(G)** Cell numbers of C2C12 and PSCs on various scaffolds over days 2, 5, 8, and 11. **(H)** CLSM images of C2C12 and PSCs on scaffolds after various culture days, with nuclei and cytoskeleton staining. CLSM images of C2C12 and PSCs on scaffolds after various culture days, with nuclei and cytoskeleton staining (L. Su et al., 2023). **(I)** Schematic diagram of the cell-cultured meat production strategy. **(J)** Cell adhesion and proliferation in GAL hydrogel (X. Wang et al., 2024). **(K)** An overview of the 3D bioprinting process. **(L)** A digital image showing the 3D printing process within a Pluronic bath. **(M)** Cell alignment and reduced F-actin were significant only in the hydrolysate-rich group, which promoted bMSC growth and muscle formation. **(N)** After 14 days of myogenic induction, the nucleus aspect ratio, FWHM of F-actin arrangement, and fusion index of bMSCs were measured. Data are presented as mean ± s.d. from triplicate experiments, with significance at *$p < 0.05$ and **$p < 0.01$. Scale bars: 75 and 100 μm (Dutta et al., 2022). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

muscle differentiation/fat differentiation medium (MADM) (Fig. 3B). Finally, cells mixed with GelMA hydrogel were proliferated for DLP printing (Fig. 3C), inducing muscle formation and fat creation through media replacement alone, achieving cultured meat with an appropriate muscle and fat ratio (Fig. 3D). This method allows the simple mixing of muscle and fat cells and the adjustment of the ratio according to preference. The successfully cultured steak-shaped meat was utilized in a basic frying recipe, confirming its potential for practical applications (Fig. 3E). In a study, prolamin scaffolds were developed for cell-based meat production (L. Su et al., 2023). CLSM images of C2C12 and PSCs on scaffolds after various culture days, with nuclei and cytoskeleton staining (Fig. 3F). After culturing, the survival and proliferation abilities of C2C12 and PSC cells were evaluated using CCK-8 analysis. C2C12 and PSC cells showed rapid proliferation and excellent biocompatibility on the prolamin scaffold, with the cell number reaching its maximum at day 11 (Fig. 3G). Evaluation of gene and protein expression levels through RT-qPCR and Western blotting revealed enhanced cell-scaffold interactions and increased expression of biomarkers associated with cell adhesion and differentiation on the prolamin scaffold. Furthermore, the mature tubular formation of C2C12 and PSC was demonstrated on the prolamin scaffold (Fig. 3H). These findings indicate the potential of the prolamin scaffold to promote cell growth and differentiation effectively.

Extrusion printing is a convenient method for producing thick tissue scaffolds using bioinks and enabling flexible control of cell types (Tibrewal, Dandekar, & Jain, 2023). Applying 3D printing to cultured meat enables commercial utilization and the cultivation of freshly extracted skeletal muscle stem cells. A 3D printable hydrogel bioink (Fig. 3I) for muscle stem cells was developed, composed of gelatin, alginate, and ε-poly-L-lysine (GAL) (X. Wang, Wang, Xu, Yin, & Hu, 2024). This bioink is based on safe and cost-effective composition and production processes, featuring high mechanical strength, stability, and easy biological activation. Biocompatibility assessment using C2C12 myoblasts and HFF-1 fibroblasts confirmed over 96.6% cell viability, demonstrating the hydrogel's suitability as a cell scaffold. Cells were well distributed on the scaffold with high survivability, supporting attachment, and maturation of muscle cells when injected or structured within the scaffold (Fig. 3J). Previously, we developed a 3D bioprinting meat cultivation platform using alginate and gelatin-based hydrogel scaffolds supplemented with plant and insect-derived biomaterials (Fig. 3K and L). In this study, the cultivation of bovine mesenchymal stem cells (bMSCs) was reported, supplemented with hydrolysates and edible insect components (Dutta et al., 2022). The results showed a significant increase in myotube formation and desmin expression, indicating that hydrolysates positively influence muscle formation (Fig. 3M). The hydrogel containing protein hydrolysates (PHs) improved cell alignment, reduced F-actin organization, and increased myotube fusion, suggesting that hydrolysates enhance muscle development (Fig. 3N).

3.1.2. Cell fiber assembly using 3D printing

Numerous studies have highlighted the crucial role of fat in determining the flavor, juiciness, and tenderness of meat (Arshad et al., 2018; Santos et al., 2021). Generally, meat with low intramuscular fat content tends to be lean and dry. In contrast, higher fat content enhances cooking flavor and significantly increases the value of meat products, such as the well-marbled Wagyu beef from Japan (Zoda et al., 2022). The fat in fresh meat primarily comes from the lipids stored within fat cells. Therefore, to accurately mimic the fat characteristics of muscle tissue in cultured meat, it is essential to co-culture muscle cells and fat cells.

A technology was developed using bovine satellite cells (bSCs) to construct tissue resembling whole-cut meat, including muscle, fat, and blood vessel cell fibers (Fig. 4A) (Kang et al., 2021). They encapsulated bovine satellite cells (bSCs) or bovine adipose-derived stem cells (bADSCs) in a mixture of Matrigel™ and fibrinogen and used vertical microextrusion bioprinting to create anisotropic tissue fibers (Fig. 4D). Since the fibers were anchored to tendon-mimicking anchors during production, this specialized biofabrication technique was called "*Tendon-Gel-Integrated Printing* (TIP)." This method helped maintain fiber structure and cell alignment (Fig. 4B and C). Under special differentiation media conditions, muscle and fat fibers upregulated differentiation markers such as MHC, PPAR-γ, and FABP-4 (Fig. 4G and H). Additionally, up to 25 fibers could be printed and cultured simultaneously (Fig. 4E and F). By bundling these fibers, they produced meat-like tissue (5 mm in diameter, 10 mm in length) that was histologically similar to real Wagyu beef steak (Fig. 4I).

4. External stimuli for muscle cell differentiation

The engineering strategies discussed in the previous section can achieve cell adhesion and alignment. Additionally, applying mechanical stimuli, which cells would experience *in vivo*, can provide further benefits for muscle cell differentiation. Specifically, mechanical stimuli such as contractile forces simulate the conditions necessary for cell proliferation, differentiation, and further muscle maturation. Besides mechanical stimuli, electrical stimulation can also be effectively used to promote cell proliferation and differentiation (K.-Y. Lee, Loh, & Wan, 2021).

4.1. Mechanical stimulation

One type of mechanical stimulation that can be applied to cell differentiation is cyclic deformation. In the human body, cyclic deformation promotes the regeneration of musculoskeletal cells due to the continuous movement of the limbs. Research has shown that cyclic deformation at frequencies below 1Hz stretches cells, leading to efficient proliferation and differentiation. Specifically, it has been demonstrated that shortening deformation is more beneficial for muscle formation compared to uniaxial deformation when the deformation amplitude is 10–15% (Somers et al., 2019). Furthermore, studies have modeled the effects of external cyclic deformation on porous cylindrical scaffolds made of fibrous materials (Yerrabelli, Somers, Grayson, & Spector, 2021). Applying cyclic deformation to cells on hydrogel scaffolds has also proven effective. For example, it was demonstrated that the light-induced cyclic bending motion of a hydrogel composed of polypeptides and graphene oxide can induce and enhance myogenic gene expression (Fig. 5A) (Chiang et al., 2021). This bending motion can be rapidly triggered in 5 s by NIR stimulation at 27–40 °C in an aquatic environment and is reversible, allowing precise control of cell behavior. Cell viability analysis showed a high relative number of cells and significant cell alignment on the cell-seeded actuator after multiple NIR stimulations (Fig. 5B and C). The alignment and proliferation of cells on the flexible, conductive, and biocompatible protein-based hydrogel actuator can be promoted by physical stimulation with low-intensity (1.2 W/cm^2) NIR laser stimulation (Fig. 5F). Additionally, mechanical loading should be applied to cells in a 3D environment that mimics the loads experienced in their natural environment. Proper 3D loading allows cells to recognize mechanical stimuli as cell-ECM interactions, facilitating accurate differentiation necessary for muscle reconstruction (M. O. Wang, Piard, Melchiorri, Dreher, & Fisher, 2015). For instance, it was reported that attaching myoblasts to gold nanorods and applying cyclic deformation can promote myogenesis by stimulating integrin receptors (Fig. 5D and E) (Ramey-Ward, Su, & Salaita, 2020).

4.2. Electrical stimulation

As the demand for mimicking native meat increases, the texture and taste of cultured meat are also gaining attention (M. Lee et al., 2024; S.-H. Lee & Choi, 2024). To produce cultured steak meat with realistic texture, large muscle tissue with densely accumulated and unidirectional aligned mature myotubes is required. Existing methods for producing bovine muscle tissue result in isotropic distribution of myotubes,

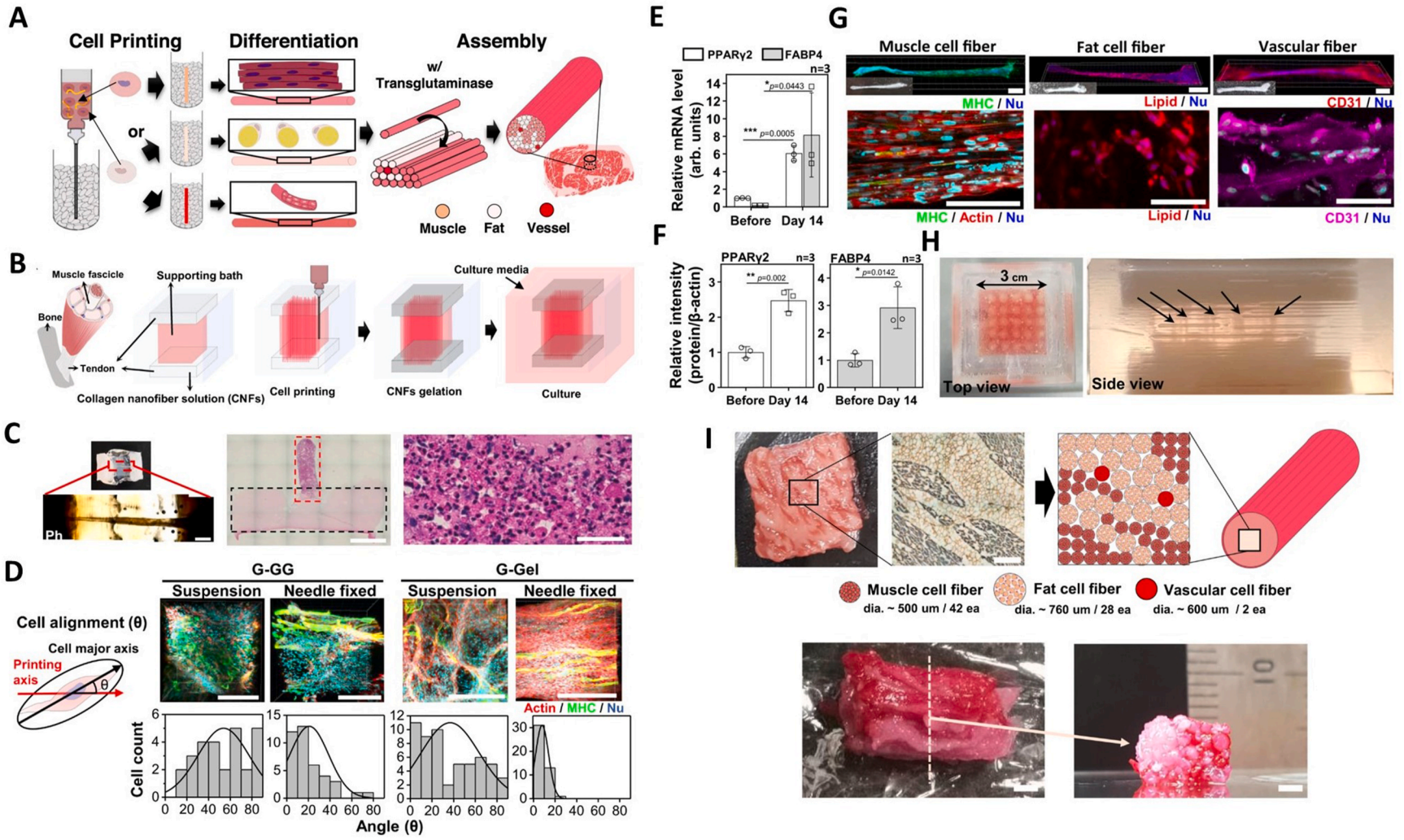


Fig. 4. **(A)** Schematic illustration of the cell fiber assembly process. **(B)** The schematic of TIP for cell printing. **(C)** Optical and phase-contrast images of bMSC tissue printed by TIP show maintained fibrous structure on day 3 after fixation, with H&E-stained images highlighting the collagen gel and fibrous tissue, scaled to 2 mm and 50 μm. **(D)** 3D-fluorescence images (upper, red: actin and green: MHC) and cell alignment measurements (lower) of the bMSC tissues printed inside G-GG and G-Gel and in suspension and needle-fixed cultures on day 3 of differentiation (after six days). **(E, F)** Relative mRNA expression levels of the *PPARγ2* and *FABP4* in TIP-derived fat tissues before and after printing at day 14 of differentiation ($n = 3$ independent samples, pairwise *t*-test comparison). **(G)** Whole fluorescence (left), optical (inset), and magnified (right) images of muscle. **(H)** Optical images of multiple tissue fabrication (25 ea.) by multiple printing. **(I)** Assembly of fibrous muscle, fat, and vascular tissues to demonstrate the proof-of-concept steak (Kang et al., 2021). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

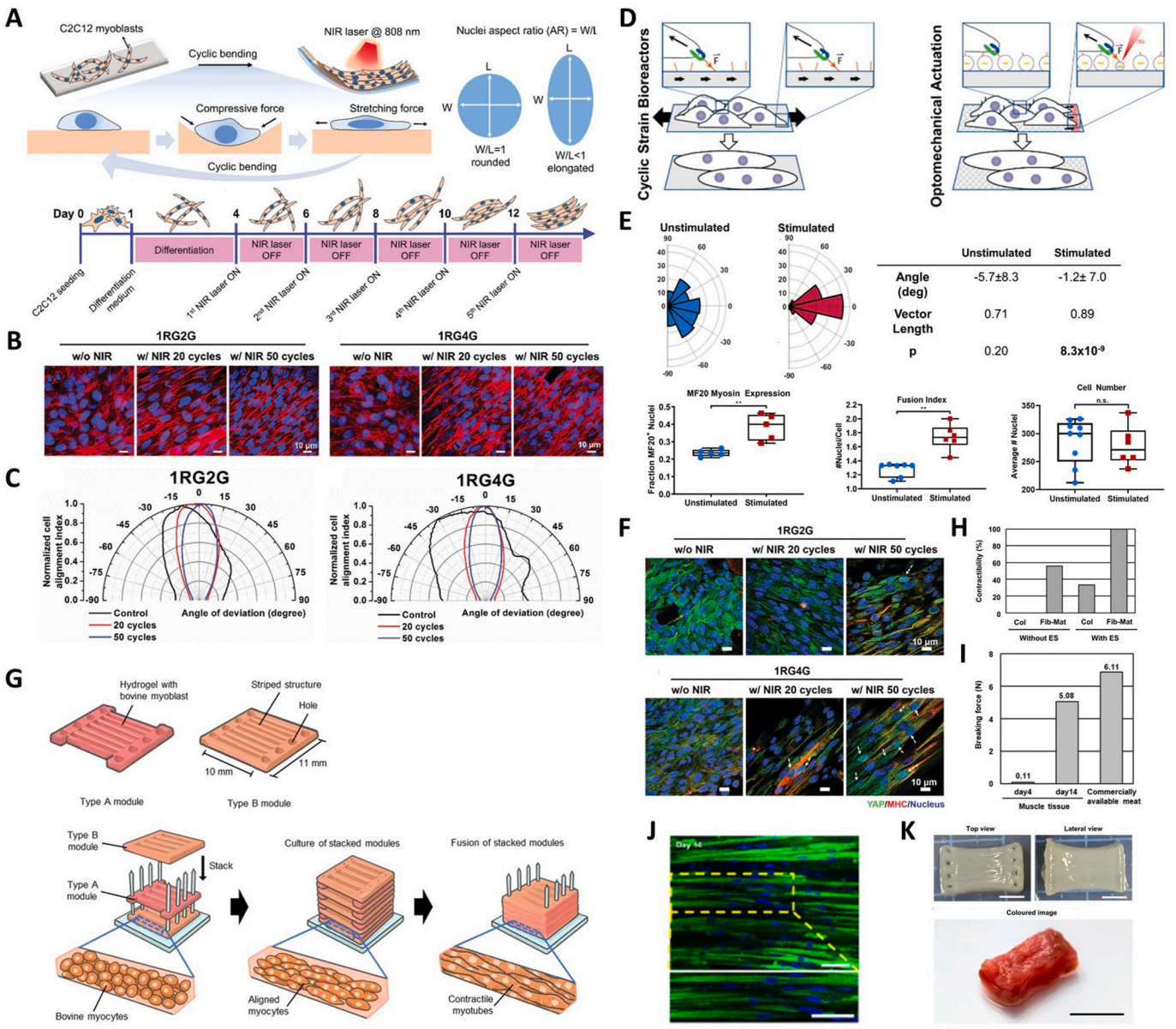


Fig. 5. **(A)** Illustration of C2C12 myoblast alignment and forces on actuators after NIR laser-induced cyclic bending. **(B)** Observations of C2C12 myoblasts on 1RG2G and 1RG4G actuators with and without NIR stimulation for 20 and 50 cycles. **(C)** Normalized alignment index versus angle of deviation for 1RG2G and 1RG4G. **(D)** Comparison of cyclic strain bioreactors promoting myogenesis through bulk force (left) versus subcellular scale using OMAs (right). **(E)** Comparison of cell orientation, MF20 positive nuclei ratio, average fusion index, and average nucleus count from three independent experiments (Ramey-Ward et al., 2020). **(F)** Immunofluorescence staining of YAP and MHC in C2C12 myoblasts on ESGRG soft actuators with and without NIR-induced cyclic bending (Chiang et al., 2021). **(G)** A diagram of the process for manufacturing millimeter-thick bovine muscle tissue. **(H)** Proportion of fibrous bovine muscle tissue contracting in response to electrical stimulation. **(I)** Breaking load of muscle tissue and commercially available beef tenderloin (n = 1). **(J)** Confocal images of bovine muscle tissue showing cell nuclei and α-actinin. **(K)** Top and lateral views of millimeter-thick bovine muscle tissue colored with red food coloring after release from pillars (Furuhashi et al., 2021). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

low density, and loss of contractility, making it difficult to use as steak meat (Li et al., 2022; Norris, Kawecki, Davis, Chen, & Rowat, 2022). However, applying electrical pulses to bovine muscle tissue can induce contraction. The effectiveness of myotube maturation depends on the frequency and amplitude of the electrical pulses (Park et al., 2024; Rao et al., 2023). For example, A new cultivation method for creating 3D cultured beef muscle tissue is proposed (Fig. 5G) (Furuhashi et al., 2021). They developed a construction method using hydrogel and electrical stimulation to form mature unidirectional aligned muscle tissue (Fig. 5H). Electrical stimulation at a frequency of 1Hz was applied to promote the growth of beef muscle (Fig. 5J). Additionally, they derived a method for manufacturing millimeter-thick beef muscle tissue with highly aligned muscle fibers using a specialized hydrogel module containing bovine myocytes (Fig. 5D). The beef muscle tissue formed under these conditions exhibited increased stiffness and low microbial contamination (Fig. 5I). The proposed method for constructing beef muscle tissue was suitable for large-scale production. Therefore, the results of this study provide a valuable technological contribution to the effective production of cultured steak meat (Fig. 5K).

4.3. Bioinspired microfluidic generation

Microfluidic technology is widely used to create templates with diverse structures and compositions. Microfibers containing cells are

particularly useful for 3D cell culture and play a significant role in muscle tissue engineering (R. Su et al., 2024). Microfibers were fabricated using a microfluidic device to support the growth and alignment of C2C12 myoblasts (Shi et al., 2015). The microfiber pattern provides a scaffold that mimics extracellular matrix, supporting cell adhesion, proliferation, and differentiation (Z. Chen et al., 2024). Their structural integrity and porosity enable enhanced nutrient diffusion and facilitate cell-cell interaction, which are crucial for the development of complex, functional tissue such as muscles. Additionally, the structure also offers improved mechanical strength ensuring the tissues maintain their shape and structural stability. Microfibers filled with various cells were demonstrated to develop into specific tissue forms, and the function of microfibers containing islet cells for diabetes treatment was investigated (Onoe et al., 2013).

Inspired by this, a coaxial microfluidic device was proposed to create core-shell structured microfibers encapsulating porcine muscle stem

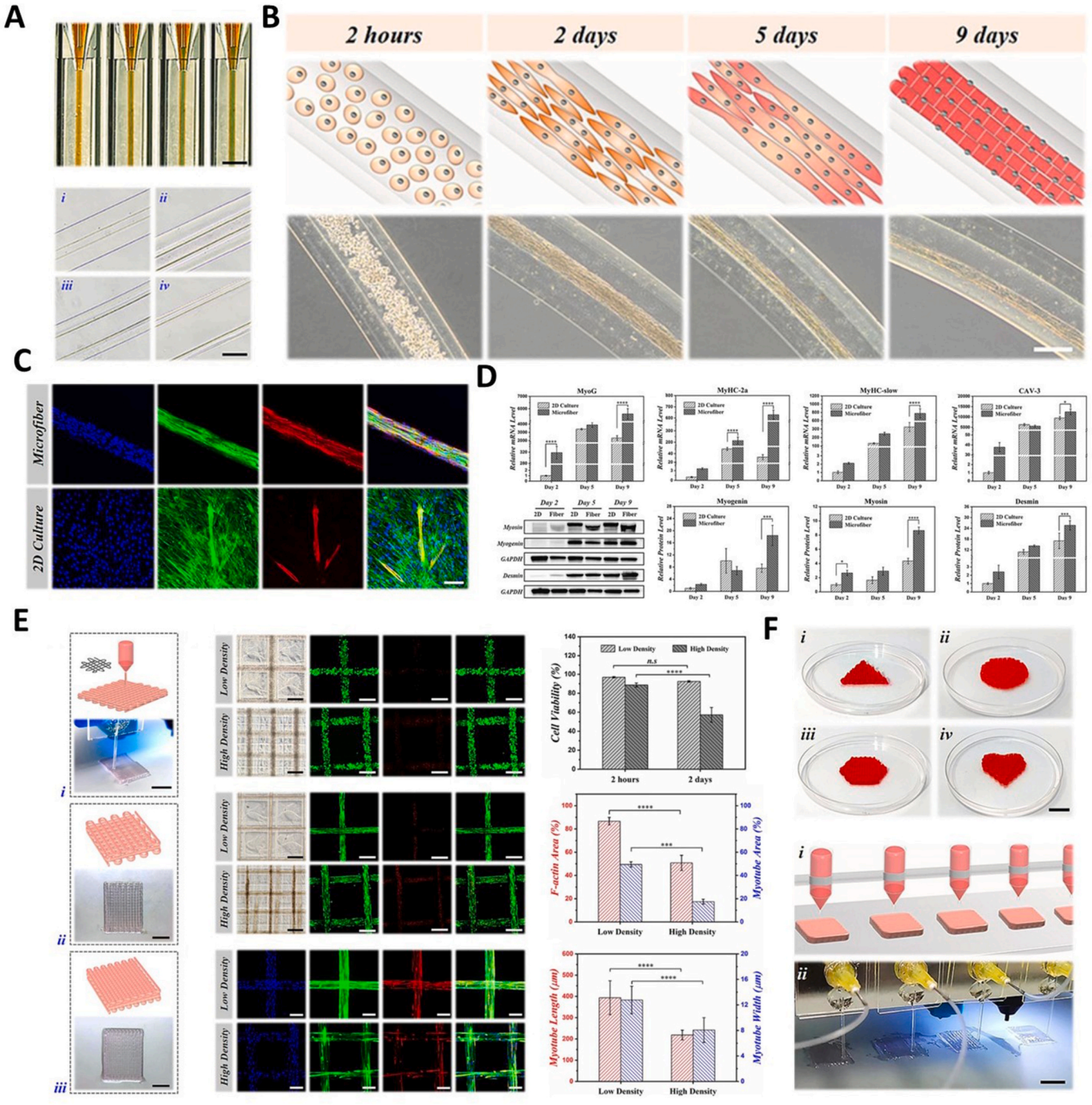


Fig. 6. **(A)** Real-time and bright field images of microfibers generated in the outer channel, showcasing core-shell structures of various sizes. **(B)** The schematic illustrations and bright field images of the life course of PMSCs **(C)** Live/dead staining images of PMSC-laden microfibers at different time points (2 h, 1 day, 2 days), showing nuclei (blue), living cells (green), dead cells (red), and merged channels (cyan). **(D)** Expression levels of myogenesis-related genes and proteins from PMSCs under microfiber and 2D culture conditions. **(E)** Microfluidic printing of bioinspired core-shell structured microfibers with varying densities (low and high). **(F)** Illustration and processing images of multi-nozzle production of cultured meat tissues, with a scale bar indicating 10 mm (Ding et al., 2023). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cells (PMSCs) (Fig. 6A and B) (Ding et al., 2023). They used sodium alginate as the shell and a hydrogel filler with cells as the core to provide an environment conducive to cell alignment (Fig. 6E). This structure promoted myogenesis and muscle protein synthesis in PMSCs, resulting in spontaneous contraction (Fig. 6C and D). These microfibers made cultured meat resemble native pork in appearance, texture, and protein composition, demonstrating promising potential for efficient and large-scale production (Fig. 6F).

4.4. Optogenetic stimulation

Opto-stimulation, for example, light, can stimulate morphogenesis and differentiation by triggering light-sensitive proteins and biomolecules. The optogenetic stimulation is reversible, i.e., when the light stimulation is inactivated, the activated cellular state is reversed. Examples of such proteins include channelrhodopsin (ChR) or halorhodopsin (HR), which undergo conformation changes in the transmembrane region of cells, modulating diverse phenotypic change (Mim, Knight, & Zartman, 2023). Inducing muscle satellites with optogenetic stimuli to differentiate into myoblast could be a potential alternative for cell-based meat production. Optogenetic stimulation (30s pulse, 2 Hz frequency) of C2C12 muscle cells with molecular transducers (Ziapin2) (Fig. 7A and B) was reported to facilitate myotube contraction-relaxation faster than normal cells, with a higher degree of fusion and enhanced viability (Fig. 7C–E) (Venturino et al., 2023). This study clearly demonstrates that myoblast maturation and contraction can be easily regulated by stimulation of the photoswitchable optogenetic proteins and biomolecules present in the muscle cells. The optogenetic stimulation platform is non-invasive, short time, and stimulation through optogenetic modulators will not modulate serious cytotoxicity to muscle cells; hence, it could be used as a potential alternative for cultivating cell-based *in vitro* meat analogs.

In this section, we discussed the four major external stimuli used for muscle cell differentiation in cultured meat production: mechanical stimulation, electrical stimulation, bioinspired microfluidic generation, and optogenetic stimulation. Each of these stimulation methods has its unique characteristics and effects, which significantly influence the quality and productivity of cultured meat. The choice of stimuli depends on the experimental conditions and production goals. To provide a clearer understanding, Table 2 compares the advantages and limitations of each stimulation method. This table will assist researchers in evaluating the suitability of each approach and in determining the optimal stimulation conditions for cultured meat production.

5. Impact to environment and global climate change

In this study, we discussed strategies of cultured meat production using advanced biofabrication tools with various biophysical stimuli. Although several breakthrough developments have been going on for cultured meat production, its impact on environment and global climate change must be taken into account. Interestingly, recent reports suggest that 80% of the global greenhouse emission (GHE) from the complex food supply chain is associated with the livestock industries, and thus, reducing the harmful impact of GHE is highly desirable as the earth grow old (Rodríguez Escobar et al., 2021). As per the United Nations Sustainable Development Goals (UNSDGs), approximately 20% of the GHE attributed to the food industry correspond to the carbon dioxide (CO_2) emission owing to the use of fossil fuels to produce the meat-based products. Understanding the fact that plants emit less carbon (CO_2 and methane) than animal individuals, the carbon footprint must be clarified for eco-friendly production of lab-grown food products (Munteanu et al., 2021). Reportedly, the water footprint associated with beef cultivation (15,400 m^3 ton^{-1}) is significantly higher than other cattle. Compared to conventional beef, the cultured meat is generally estimated a lower carbon footprint value depending on its production procedure (Dupont, Harms, & Fiebelkorn, 2022; Rodríguez Escobar et al., 2021). The most

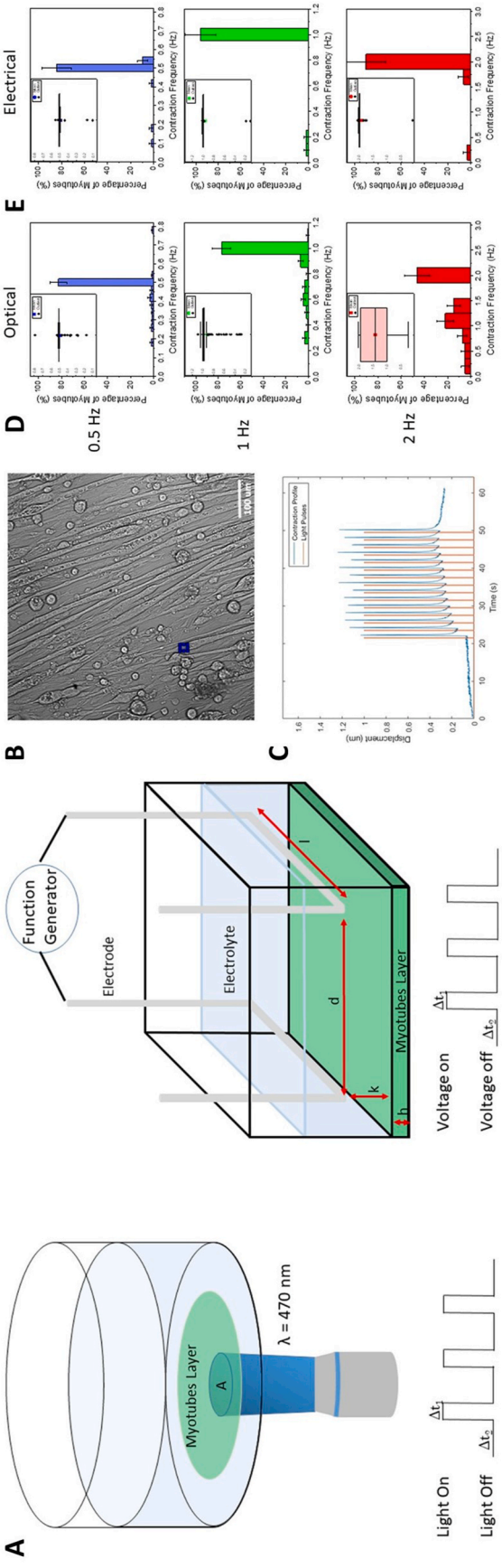


Fig. 7. Optogenetic stimulation of muscle cells for modulating myogenesis. **(A)** Schematic illustration of the setup of the optogenetic stimulation system. **(B)** Bright-field image of the stimulated C2C12 cells showing the contraction under optogenetic stimulation for 30s. Scale bar: 100 μm **(C)** The mean contraction path (blue line) of the optogenetic stimulation derived from the ROI bright-field image of C2C12 cells. **(D)** Histogram of mean contraction of the myotubes stimulated with light pulse 30s, 1 and 2 Hz frequency. **(E)** The histogram showing the mean contraction of myotubes under optoelectric stimulation with 30s pulse, 1 and 2 Hz frequency (Venturino et al., 2023). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Comparative analysis of external stimuli for muscle cell differentiation towards cultured meat production.

Stimulus	Cell Types	Stimulation Method	Outcome	Advantages	Limitations	Ref.
Mechanical stimulation	Mouse + human iPSC-CMs	5~10%, 1~2Hz	Improved CM survival, metabolism, sarcomere alignment, and contraction force generation.	Enhanced tissue maturation through physiological remodeling, Achieved contraction force comparable to native myocardium.	Still requires further optimization for human clinical application, Lower force compared to native human myocardium.	Jahanian, Ramirez, and O'Hara (2024)
Electrical stimulation	NRCMs	2 ms pulses 4 V/cm, 1 Hz	Increased CM volume fraction, elongation, connexin-43 expression	Myocyte geometry and gap junction formation resemble native myocardium, Improved sarcomere alignment, Enhanced electrical properties	Functional gap junction formation not confirmed, Additional functional analyses (*e.g.*, conduction velocity) needed	Lasher, Pahnke, Johnson, Sachse, and Hitchcock (2012)
Bioinspired microfluidic generation	C2C12, NIH-3 T3	Development of helical micromotors using microfluidic technology	Promote adhesion, proliferation, and migration of muscle cells, Cells can assemble into tissue-like structures	Capable of forming complex tissue structures	Limited research on encapsulation of cells inside micromotors	Zhuge et al. (2022)
Optogenetic stimulation	C2C12 myoblasts upconversion nanoparticles	blue light (455 nm and 10–80 mW/cm^2) for 30, 60s	NIR stimulation induced greater Ca^{2+} influx compared to direct blue light	Deeper tissue penetration with NIR light, Remote control capability	Requires optimized UCNP synthesis and binding, UCNP density needs fine-tuning for efficiency	Maemura, Le, Takahashi, Matsumura, and Maenosono (2023)

prevalent factors affecting the carbon footprint value of cultured meat production lies on the energy used (*e.g.*, use of growth medium and bioreactors), raw materials involved (*e.g.*, culture in FBS or any alternatives, plant-based nutrients, and fossil fuels), production scale (*e.g.*, laboratory scale or industrial scale), and its transportation by vehicles. Table 3 listed a relative comparison of carbon footprint data between conventional vs. cultured meat.

Although the carbon footprint in cultured meat is lower than the conventional beef meat, it is important to access the energy consumption value for meat production. This includes the design and development of various stimuli-responsive bioreactors for enhancing the muscle cell growth and differentiation than conventional bioreactors in a little space with time savings. The more time required to produce the lab-grown meat, the more carbon emission is required. Looking forward, we hope that 3D bioprinting with smart stimuli-assisted bioreactor system will not only minimize the production time but also impact on less GHE in the future.

6. Conclusion and remarks

In conclusion, cultured meat technology offers a transformative solution to address the environmental, ethical, and health issues associated with traditional meat production. Significant progress has been made in developing the processes necessary to produce muscle tissue *in vitro*, including advancements in cell culture techniques, scaffold engineering, and bioreactor systems. Innovations such as mechanical and electrical stimulation, along with 3D printing technology, have further enhanced the quality and complexity of cultured meat products, making them more comparable to conventional meat. However, significant hurdles remain before widespread adoption becomes feasible. One major challenge is the high production cost, which can be mitigated through research into cost-effective growth media, optimized nutrient delivery systems, and energy-efficient bioreactors. Scaling production from laboratory to industrial levels poses technical challenges such as ensuring consistent tissue quality and efficient bioreactor performance. Overcoming these issues will require innovations in bioreactor design, automation, and continuous monitoring systems. Integrating technologies like 3D printing and mechanical stimulation into large-scale processes will also necessitate further refinement to maintain efficiency and product quality. Regulatory approval remains a significant hurdle due to the need for rigorous safety assessments and compliance with diverse regional standards. Unlike conventional meat, cultured meat involves novel production methods that raise unique regulatory questions related to cell sourcing, growth media, and processing techniques. To navigate this, close collaboration between industry leaders, regulatory agencies, and scientific experts is essential to establish clear guidelines and safety protocols. For example, frameworks similar to those established in Singapore, the first country to approve the sale of cultured meat, can serve as models for other regions. Transparent documentation of production processes, safety data, and third-party validation will help streamline approvals and build confidence among regulatory bodies. Additionally, harmonizing standards across international markets will facilitate smoother global adoption. Equally important is consumer acceptance, which hinges on overcoming skepticism and misconceptions surrounding cultured meat. Many consumers express concerns about the safety, taste, nutritional value, and ethical implications of lab-grown meat. Effective strategies to address these concerns include educational campaigns highlighting the benefits of cultured meat, such as its potential to reduce greenhouse gas emissions, conserve water, and eliminate animal suffering. Public demonstrations, media outreach, and transparent communication of the production process can help demystify cultured meat and emphasize its safety and sustainability. Moreover, achieving sensory and nutritional parity with traditional meat is crucial for consumer acceptance. Investments in flavor, texture, and nutritional optimization are necessary to ensure cultured meat meets or exceeds consumer expectations. Offering products in familiar formats, such as burgers, nuggets, and sausages, can also ease the transition. Partnering with well-known food brands and chefs to introduce cultured meat in trusted settings may further accelerate acceptance. Building trust and transparency through labeling practices that clearly convey how cultured meat is produced and its benefits can also reduce hesitation. Surveys indicate that consumers are more likely to accept cultured meat if they understand its environmental and ethical advantages. Involving the public in the conversation through taste trials, feedback sessions, and community outreach can create a sense of inclusion and ownership.

Ultimately, addressing all the above mentioned challenges will require a multi-stakeholder approach involving researchers, policymakers, industry leaders, and the public. Continued investment, innovation, and transparent communication are essential to overcoming these barriers and ensuring that cultured meat becomes a viable,

Table 3
Comparative analysis of carbon footprint with conventional meat vs. cultured meat (Munteanu et al., 2021).

Type of meat	Carbon footprint	Impact on environment
Conventional beef meat	27–60 kg CO_2 emission/kg	High GHE
Cultured beef meat	1–7 kg CO_2 emission/kg	Low GHE

accepted component of the global food system, contributing to a more sustainable and ethical future for meat production.

7. Author contributions

Conceptualization, methodology, formal analysis, validation, investigation: J.L., S.D.D., and T.V.P.; Data curation, software: S.D.D.; Writing-Original draft; J.L. and S.D.D.; Writing-Review & Editing: T.V.P, S.J.C. and K.T.L.; Visualization, Supervision: S.J.C. and K.T.L.; Project administration, Funding acquisition: S.J.C. and K.T.L.

Acknowledgments

This study was supported by the 'Basic Science Research Program' through the 'National Research Foundation of Korea' funded by the 'Ministry of Education' (NRF-2018R1A16A1A03025582 and NRF2022R1I1A3063302). This research was supported by the MSIT (Ministry of Science and ICT), Korea, under the Innovative Human Resource Development for Local Intellectualization support program (IITP-2024-RS-2023-00260267) supervised by the IITP (Institute for Information & communications Technology Planning & Evaluation.

Data availability

Data will be made available on request.

References

Acevedo, C. A., Orellana, N., Avarias, K., Ortiz, R., Benavente, D., & Prieto, P. (2018). Micropatterning technology to design an edible film for in vitro meat production. *Food and Bioprocess Technology, 11*, 1267–1273.

Akram, Z. (2024). Culturing of animal cells, stem cells, tissues, and Organs for industrial products: An introduction. In *Recent advances in industrial biochemistry* (pp. 399–418). Springer.

Alam, A. N., Kim, C.-J., Kim, S.-H., Kumari, S., Lee, E.-Y., Hwang, Y.-H., et al. (2024). Scaffolding fundamentals and recent advances in sustainable scaffolding techniques for cultured meat development. *Food Research International, 114549*.

Amirvaresi, A., & Ovissipour, R. (2024). Evaluating the efficacy of serum-free media supplemented with protein isolates for bovine satellite cell proliferation: A sustainable approach for cultivated meat production. *bioRxiv*, 2024.08.23.609451.

Arshad, M. S., Javed, M., Sohaib, M., Saeed, F., Imran, A., & Amjad, Z. (2017). Tissue engineering approaches to develop cultured meat from cells: A mini review. *Cogent Food & Agriculture, 3*, Article 1320814.

Arshad, M. S., Sohaib, M., Ahmad, R. S., Nadeem, M. T., Imran, A., Arshad, M. U., et al. (2018). Ruminant meat flavor influenced by different factors with special reference to fatty acids. *Lipids in Health and Disease, 17*, 1–13.

Asakura, A., Rudnicki, M. A., & Komaki, M. (2001). Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. *Differentiation, 68*, 245–253.

Asakura, A., Seale, P., Girgis-Gabardo, A., & Rudnicki, M. A. (2002). Myogenic specification of side population cells in skeletal muscle. *The Journal of cell biology, 159*, 123–134.

Baig, M. A., Ajayi, F. F., Hamdi, M., Baba, W., Brishti, F. H., Khalid, N., et al. (2024). Recent research advances in meat analogues: A comprehensive review on production, protein sources, quality attributes, analytical techniques used, and consumer perception. *Food Reviews International*, 1–32.

Banan Sadeghian, R., Ebrahimi, M., & Salehi, S. (2018). Electrical stimulation of microengineered skeletal muscle tissue: Effect of stimulus parameters on myotube contractility and maturation. *Journal of Tissue Engineering and Regenerative Medicine, 12*, 912–922.

Barbosa, W., Correia, P., Vieira, J., Leal, I., Rodrigues, L., Nery, T., et al. (2023). Trends and technological challenges of 3D bioprinting in cultured meat: Technological prospection. *Applied Sciences, 13*, Article 12158.

Ben-Arye, T., Shandalov, Y., Ben-Shaul, S., Landau, S., Zagury, Y., Ianovici, I., et al. (2020). Textured soy protein scaffolds enable the generation of three-dimensional bovine skeletal muscle tissue for cell-based meat. *Nature Food, 1*, 210–220.

Bhat, Z. F., Morton, J. D., Mason, S. L., Bekhit, A. E. D. A., & Bhat, H. F. (2019). Technological, regulatory, and ethical aspects of in vitro meat: A future slaughter-free harvest. *Comprehensive Reviews in Food Science and Food Safety, 18*, 1192–1208.

Bjare, U. (1992). Serum-free cell culture. *Pharmacology & therapeutics, 53*, 355–374.

Bomkamp, C., Skaalure, S. C., Fernando, G. F., Ben-Arye, T., Swartz, E. W., & Specht, E. A. (2022). Scaffolding biomaterials for 3D cultivated meat: Prospects and challenges. *Advanced Science, 9*, Article 2102908.

Boonen, K. J., Langelaan, M. L., Polak, R. B., van der Schaft, D. W., Baaijens, F. P., & Post, M. J. (2010). Effects of a combined mechanical stimulation protocol: Value for skeletal muscle tissue engineering. *Journal of Biomechanics, 43*, 1514–1521.

Boukid, F. (2021). Plant-based meat analogues: From niche to mainstream. *European food research and technology, 247*, 297–308.

Cai, S., Wu, C., Yang, W., Liang, W., Yu, H., & Liu, L. (2020). Recent advance in surface modification for regulating cell adhesion and behaviors. *Nanotechnology Reviews, 9*, 971–989.

Cedillo-Servin, G., Dahri, O., Meneses, J., van Duijn, J., Moon, H., Sage, F., et al. (2024). 3D printed magneto-active microfiber scaffolds for remote stimulation and guided organization of 3D in vitro skeletal muscle models. *Small, 20*, Article 2307178.

Chandrababu, A., & Puthumana, J. (2024). CRISPR-edited, cell-based future-proof meat and seafood to enhance global food security and nutrition. *Cytotechnology*, 1–34.

Chen, Z., Du, C., Liu, S., Liu, J., Yang, Y., Dong, L., et al. (2024). Progress in biomaterials inspired by the extracellular matrix. *Giant*, Article 100323.

Chen, Y. P., Feng, X., Blank, I., & Liu, Y. (2022). Strategies to improve meat-like properties of meat analogs meeting consumers' expectations. *Biomaterials, 287*, Article 121648.

Chiang, M.-Y., Lo, Y.-C., Lai, Y.-H., Yong, Y.-Y. A., Chang, S.-J., Chen, W.-L., et al. (2021). Protein-based soft actuator with high photo-response and easy modulation for anisotropic cell alignment and proliferation in a liquid environment. *Journal of Materials Chemistry B, 9*, 6634–6645.

Choi, H., & Myung, K. (2014). Calcitriol enhances fat synthesis factors and calpain activity in co-cultured cells. *Cell Biology International, 38*, 910–917.

Chung, S., & King, M. W. (2011). Design concepts and strategies for tissue engineering scaffolds. *Biotechnology and Applied Biochemistry, 58*, 423–438.

David, S., Tsukerman, A., Safina, D., Maor-Shoshani, A., Lavon, N., & Levenberg, S. (2023). Co-culture approaches for cultivated meat production. *Nature Reviews Bioengineering, 1*, 817–831.

de Mello, A. F. M., de Souza Vandenberghe, L. P., Herrmann, L. W., Letti, L. A. J., Burgos, W. J. M., Scapini, T., et al. (2024). Strategies and engineering aspects on the scale-up of bioreactors for different bioprocesses. *Systems Microbiology and Biomanufacturing, 4*, 365–385.

Dick, A., Bhandari, B., & Prakash, S. (2019). 3D printing of meat. *Meat Science, 153*, 35–44.

Ding, X., Zhuge, W., Zhang, Y., Ding, S., Wang, J., & Zhou, G. (2023). Microfluidic generation of bioinspired core–shell structured microfibers for cultured meat. *Chemical Engineering Journal, 478*, Article 147467.

Dupont, J., Harms, T., & Fiebelkorn, F. (2022). Acceptance of cultured meat in Germany—application of an extended theory of planned behaviour. *Foods, 11*, 424.

Dutta, S. D., Ganguly, K., Jeong, M.-S., Patel, D. K., Patil, T. V., Cho, S.-J., et al. (2022). Bioengineered lab-grown meat-like constructs through 3D bioprinting of antioxidative protein hydrolysates. *ACS applied materials & interfaces, 14*, 34513–34526.

Edelman, P., McFarland, D., Mironov, V., & Matheny, J. (2005). Commentary: In vitro-cultured meat production. *Tissue Engineering, 11*, 659–662.

Elemoso, A., Shalunov, G., Balakhovsky, Y. M., Ostrovskiy, A. Y., & Khesuani, Y. D. (2020). 3D bioprinting: The roller coaster ride to commercialization. *International Journal of Bioprinting, 6*.

Enrione, J., Blaker, J. J., Brown, D. I., Weinstein-Oppenheimer, C. R., Pepczynska, M., Olguín, Y., et al. (2017). Edible scaffolds based on non-mammalian biopolymers for myoblast growth. *Materials, 10*, 1404.

Fasciano, S., Wheba, A., Ddamulira, C., & Wang, S. (2024). Recent advances in scaffolding biomaterials for cultivated meat. *Biomaterials Advances, 213897*.

Filippi, M., Buchner, T., Yasa, O., Weirich, S., & Katzschmann, R. K. (2022). Microfluidic tissue engineering and bio-actuation. *Advanced Materials, 34*, Article 2108427.

Furuhashi, M., Morimoto, Y., Shima, A., Nakamura, F., Ishikawa, H., & Takeuchi, S. (2021). Formation of contractile 3D bovine muscle tissue for construction of millimetre-thick cultured steak. *Npj Science of Food, 5*, 6.

Galanakis, C. M. (2024). The future of food. *Foods, 13*, 506.

Godfray, H. C. J., Aveyard, P., Garnett, T., Hall, J. W., Key, T. J., Lorimer, J., et al. (2018). Meat consumption, health, and the environment. *Science, 361*, Article eaam5324.

Gome, G., Chak, B., Tawil, S., Shpatz, D., Giron, J., Brajzblat, I., et al. (2024). Cultivation of bovine mesenchymal stem cells on plant-based scaffolds in a macrofluidic single-use bioreactor for cultured meat. *Foods, 13*, 1361.

Guo, X., Wang, D., He, B., Hu, L., & Jiang, G. (2024). 3D bioprinting of cultured meat: A promising avenue of meat production. *Food and Bioprocess Technology, 17*, 1659–1680.

Hanan, F. A., Karim, S. A., Aziz, Y. A., Ishak, F. A. C., & Sumarjan, N. (2024). Consumer's cultured meat perception and acceptance determinants: A systematic review and future research agenda. *International Journal of Consumer Studies, 48*, Article e13088.

Heidarian, P., Kouzani, A. Z., Kaynak, A., Paulino, M., Nasri-Nasrabadi, B., Zolfagharian, A., et al. (2020). Dynamic plant-derived polysaccharide-based hydrogels. *Carbohydrate polymers, 231*, Article 115743.

Hill, A. B. T., Bressan, F. F., Murphy, B. D., & Garcia, J. M. (2019). Applications of mesenchymal stem cell technology in bovine species. *Stem Cell Research & Therapy, 10*, 1–13.

Hossner, K., Yemm, R., Vierck, J., & Dodson, M. (1997). Insulin-like growth factor (IGF)-I and-II and IGFBP secretion by ovine satellite cell strains grown alone or in coculture with 3T3-L1 preadipocytes. *In Vitro Cellular & Developmental Biology-Animal, 33*, 791–795.

Humbird, D. (2021). Scale-up economics for cultured meat. *Biotechnology and Bioengineering, 118*, 3239–3250.

Ismail, I., Hwang, Y.-H., & Joo, S.-T. (2020). Meat analog as future food: A review. *Journal Of Animal Science And Technology, 62*, 111.

Jahanian, A., Ramirez, J., & O'Hara, I. (2024). Advancing precision fermentation: Minimizing power demand of industrial scale bioreactors through mechanistic modelling. *Computers & Chemical Engineering*, Article 108755.

Jankovic, M. G., Stojkovic, M., Bojic, S., Jovicic, N., Kovacevic, M. M., Ivosevic, Z., et al. (2023). Scaling up human mesenchymal stem cell manufacturing using bioreactors for clinical uses. *Current Research in Translational Medicine, 71*, Article 103393.
Jeong, D., Seo, J. W., Lee, H. G., Jung, W. K., Park, Y. H., & Bae, H. (2022). Efficient myogenic/adipogenic transdifferentiation of bovine fibroblasts in a 3D bioprinting system for steak-type cultured meat production. *Advanced Science, 9*, Article 2202877.
Jin, Y.-R., Liu, Y., & Jiang, F.-L. (2021). Positive sorption behaviors in the ligand exchanges for water-soluble quantum dots and a strategy for specific targeting. *ACS applied materials & interfaces, 13*, 51746–51758.
Johnson, A. P., Sabu, C., Nivitha, K., Sankar, R., Shirin, V. A., Henna, T., et al. (2022). Bioinspired and biomimetic micro-and nanostructures in biomedicine. *Journal of Controlled Release, 343*, 724–754.
Kadim, I. T., Mahgoub, O., Baqir, S., Faye, B., & Purchas, R. (2015). Cultured meat from muscle stem cells: A review of challenges and prospects. *Journal of Integrative Agriculture, 14*, 222–233.
Kang, D.-H., Louis, F., Liu, H., Shimoda, H., Nishiyama, Y., Nozawa, H., et al. (2021). Engineered whole cut meat-like tissue by the assembly of cell fibers using tendon-gel integrated bioprinting. *Nature Communications, 12*, 5059.
Kasani, A. A., Esmaeili, A., & Golzary, A. (2022). Software tools for microalgae biorefineries: Cultivation, separation, conversion process integration, modeling, and optimization. *Algal Research, 61*, Article 102597.
Kawecki, N. S., Norris, S. C., Xu, Y., Wu, Y., Davis, A. R., Fridman, E., et al. (2023). Engineering multicomponent tissue by spontaneous adhesion of myogenic and adipogenic microtissues cultured with customized scaffolds. *Food Research International, 172*, Article 113080.
Khodabukus, A., Madden, L., Prabhu, N. K., Koves, T. R., Jackman, C. P., Muoio, D. M., et al. (2019). Electrical stimulation increases hypertrophy and metabolic flux in tissue-engineered human skeletal muscle. *Biomaterials, 198*, 259–269.
Kim, C.-J., Kim, S.-H., Lee, E.-Y., Hwang, Y.-H., Lee, S.-Y., & Joo, S.-T. (2024). Effect of chicken age on proliferation and differentiation abilities of muscle stem cells and nutritional characteristics of cultured meat tissue. *Food Science of Animal Resources, 44*, 1167.
Kolkmann, A., Post, M., Rutjens, M., Van Essen, A., & Moutsatsou, P. (2020). Serum-free media for the growth of primary bovine myoblasts. *Cytotechnology, 72*, 111–120.
Kumar, A., Sood, A., & Han, S. S. (2023). Technological and structural aspects of scaffold manufacturing for cultured meat: Recent advances, challenges, and opportunities. *Critical Reviews in Food Science and Nutrition, 63*, 585–612.
Lasher, R. A., Pahnke, A. Q., Johnson, J. M., Sachse, F. B., & Hitchcock, R. W. (2012). Electrical stimulation directs engineered cardiac tissue to an age-matched native phenotype. *Journal of Tissue Engineering, 3*, Article 2041731412455354.
Lee, S.-H., & Choi, J. (2024). Three-dimensional scaffolds, materials, and fabrication for cultured meat applications: A scoping review and future direction. *Food Hydrocolloids*, Article 109881.
Lee, H., Kim, S. H., Lee, J. S., Lee, Y. J., Lee, O. J., Ajiteru, O., et al. (2023). Functional skeletal muscle regeneration using muscle mimetic tissue fabricated by microvalve-assisted coaxial 3D bioprinting. *Advanced Healthcare Materials, 12*, Article 2202664.
Lee, K.-Y., Loh, H.-X., & Wan, A. C. (2021). Systems for muscle cell differentiation: From bioengineering to future food. *Micromachines, 13*, 71.
Lee, M., Park, S., Choi, B., Choi, W., Lee, H., Lee, J. M., et al. (2024). Cultured meat with enriched organoleptic properties by regulating cell differentiation. *Nature Communications, 15*, 77.
Lee, S. Y., Yun, S. H., Jeong, J. W., Kim, J. H., Kim, H. W., Choi, J. S., et al. (2022). Review of the current research on fetal bovine serum and the development of cultured meat. *Food Science of Animal Resources, 42*, 775.
Leung, A. K.-y., Chong, M., Fernandez, T. M., & Ng, S. T. (2023). Higher well-being individuals are more receptive to cultivated meat: An investigation of their reasoning for consuming cultivated meat. *Appetite, 184*, Article 106496.
Levi, S., Yen, F.-C., Baruch, L., & Machluf, M. (2022). Scaffolding technologies for the engineering of cultured meat: Towards a safe, sustainable, and scalable production. *Trends in Food Science & Technology, 126*, 13–25.
Lew, E. T., Yuen Jr, J. S., Zhang, K. L., Fuller, K., Frost, S. C., & Kaplan, D. L. (2024). Chemical and sensory analyses of cultivated pork fat tissue as a flavor enhancer for meat alternatives. *Scientific Reports, 14*, Article 17643.
Li, C.-H., Yang, I.-H., Ke, C.-J., Chi, C.-Y., Matahum, J., Kuan, C.-Y., et al. (2022). The production of fat-containing cultured meat by stacking aligned muscle layers and adipose layers formed from gelatin-soymilk scaffold. *Frontiers in Bioengineering and Biotechnology, 10*, Article 875069.
Lim, D., Renteria, E. S., Sime, D. S., Ju, Y. M., Kim, J. H., Criswell, T., et al. (2022). Bioreactor design and validation for manufacturing strategies in tissue engineering. *Bio-design and manufacturing, 5*, 43–63.
Liu, P., Song, W., Bassey, A. P., Tang, C., Li, H., Ding, S., et al. (2023). Preparation and quality evaluation of cultured fat. *Journal of Agricultural and Food Chemistry, 71*, 4113–4122.
Machovina, B., Feeley, K. J., & Ripple, W. J. (2015). Biodiversity conservation: The key is reducing meat consumption. *Science of The Total Environment, 536*, 419–431.
Maemura, D., Le, T. S., Takahashi, M., Matsumura, K., & Maenosono, S. (2023). Optogenetic calcium ion influx in myoblasts and myotubes by near-infrared light using upconversion nanoparticles. *ACS applied materials & interfaces, 15*, 42196–42208.
Mancini, M. C., & Antonioli, F. (2022). The future of cultured meat between sustainability expectations and socio-economic challenges. In *Future foods* (pp. 331–350). Elsevier.
Manzoki, M. C., de Mello, A. F. M., Martinez-Burgos, W. J., da Silva Vale, A., Biagini, G., Piazenski, I. N., et al. (2024). Scaling-up of cultivated meat production process. In *Cultivated meat: Technologies, commercialization and challenges* (pp. 241–264). Springer.
Martin, Y., & Vermette, P. (2005). Bioreactors for tissue mass culture: Design, characterization, and recent advances. *Biomaterials, 26*, 7481–7503.
Messmer, T., Klevernic, I., Furquim, C., Ovchinnikova, E., Dogan, A., Cruz, H., et al. (2022). A serum-free media formulation for cultured meat production supports bovine satellite cell differentiation in the absence of serum starvation. *Nature Food, 3*, 74–85.
Mim, M. S., Knight, C., & Zartman, J. J. (2023). Quantitative insights in tissue growth and morphogenesis with optogenetics. *Physical Biology, 20*, Article 061001.
Mueller, C., Trujillo-Miranda, M., Maier, M., Heath, D. E., O'Connor, A. J., & Salehi, S. (2021). Effects of external stimulators on engineered skeletal muscle tissue maturation. *Advanced Materials Interfaces, 8*, Article 2001167.
Munteanu, C., Mireşan, V., Răducu, C., Ihuţ, A., Uiuiu, P., Pop, D., et al. (2021). Can cultured meat be an alternative to farm animal production for a sustainable and healthier lifestyle? *Frontiers in Nutrition, 8*, Article 749298.
Nikkhah, A., Rohani, A., Zarei, M., Kulkarni, A., Batarseh, F. A., Blackstone, N. T., et al. (2023). Toward sustainable culture media: Using artificial intelligence to optimize reduced-serum formulations for cultivated meat. *Science of the Total Environment, 894*, Article 164988.
Norris, S. C., Kawecki, N. S., Davis, A. R., Chen, K. K., & Rowat, A. C. (2022). Emulsion-templated microparticles with tunable stiffness and topology: Applications as edible microcarriers for cultured meat. *Biomaterials, 287*, Article 121669.
O'Neill, E. N., Cosenza, Z. A., Baar, K., & Block, D. E. (2021). Considerations for the development of cost-effective cell culture media for cultivated meat production. *Comprehensive Reviews in Food Science and Food Safety, 20*, 686–709.
Onoe, H., Okitsu, T., Itou, A., Kato-Negishi, M., Gojo, R., Kiriya, D., et al. (2013). Metre-long cell-laden microfibres exhibit tissue morphologies and functions. *Nature Materials, 12*, 584–590.
Orellana, N., Sánchez, E., Benavente, D., Prieto, P., Enrione, J., & Acevedo, C. A. (2020). A new edible film to produce in vitro meat. *Foods, 9*, 185.
Palladino, F., Marcelino, P. R. F., Schlogl, A. E., José, Á. H. M., Rodrigues, R.d. C. L. B., Fabrino, D. L., et al. (2024). Bioreactors: Applications and innovations for a sustainable and healthy future—a critical review. *Applied Sciences, 14*, 9346.
Park, S., Hong, Y., Park, S., Kim, W., Gwon, Y., Sharma, H., et al. (2024). Engineering considerations on large-scale cultured meat production. *Tissue Engineering Part B: Reviews, 30*, 423–435.
Pasitka, L., Wissotsky, G., Ayyash, M., Yarza, N., Rosoff, G., Kaminker, R., et al. (2024). Empirical economic analysis shows cost-effective continuous manufacturing of cultivated chicken using animal-free medium. *Nature Food, 5*, 693–702.
Peng, H., & Huard, J. (2004). Muscle-derived stem cells for musculoskeletal tissue regeneration and repair. *Transplant Immunology, 12*, 311–319.
Pentidis, N. (2024). *Effects of maturation and exercise loading on the adaptation process of morphological and mechanical properties of muscles and tendons in prepubertal children*.
Plikus, M. V., Wang, X., Sinha, S., Forte, E., Thompson, S. M., Herzog, E. L., et al. (2021). Fibroblasts: Origins, definitions, and functions in health and disease. *Cell, 184*, 3852–3872.
Poetsch, M. S., Strano, A., & Guan, K. (2022). Human induced pluripotent stem cells: From cell origin, genomic stability, and epigenetic memory to translational medicine. *Stem cells, 40*, 546–555.
Portanguen, S., Tournayre, P., Sicard, J., Astruc, T., & Mirade, P.-S. (2019). Toward the design of functional foods and biobased products by 3D printing: A review. *Trends in Food Science & Technology, 86*, 188–198.
Post, M. J. (2014). Cultured beef: Medical technology to produce food. *Journal of the Science of Food and Agriculture, 94*, 1039–1041.
Post, M. J., Levenberg, S., Kaplan, D. L., Genovese, N., Fu, J., Bryant, C. J., et al. (2020). Scientific, sustainability and regulatory challenges of cultured meat. *Nature Food, 1*, 403–415.
Post, M., & Van Der Weele, C. (2020). Principles of tissue engineering for food. In *Principles of tissue engineering* (pp. 1355–1368). Elsevier.
Purslow, P. P. (2018). Contribution of collagen and connective tissue to cooked meat toughness; some paradigms reviewed. *Meat Science, 144*, 127–134.
Quek, J. P., Gaffoor, A. A., Tan, Y. X., Tan, T. R. M., Chua, Y. F., Leong, D. S. Z., et al. (2024). Exploring cost reduction strategies for serum free media development. *Npj Science of Food, 8*, 107.
Ramey-Ward, A. N., Su, H., & Salaita, K. (2020). Mechanical stimulation of adhesion receptors using light-responsive nanoparticle actuators enhances myogenesis. *ACS applied materials & interfaces, 12*, 35903–35917.
Rao, K. M., Choi, S. M., & Han, S. S. (2023). A review on directional muscle cell growth in scaffolding biomaterials with aligned porous structures for cultivated meat production. *Food Research International, 168*, Article 112755.
Ravanbakhsh, H., Karamzadeh, V., Bao, G., Mongeau, L., Juncker, D., & Zhang, Y. S. (2021). Emerging technologies in multi-material bioprinting. *Advanced materials, 33*, Article 2104730.
Reid, Y., Storts, D., Riss, T., & Minor, L. (2013). *Authentication of human cell lines by STR DNA profiling analysis*.
Rodríguez Escobar, M. I., Cadena, E., Nhu, T. T., Cooreman-Algoed, M., De Smet, S., & Dewulf, J. (2021). Analysis of the cultured meat production system in function of its environmental footprint: Current status, gaps and recommendations. *Foods, 10*, 2941.
Roy, N. K., Panda, S., & Dey, G. (2023). Engineering a sustainable protein revolution: Recent advances in cultured meat production. *Food Bioengineering, 2*, 301–316.
Rubio, N. R., Xiang, N., & Kaplan, D. L. (2020). Plant-based and cell-based approaches to meat production. *Nature Communications, 11*, 1–11.

Santos, D., Monteiro, M. J., Voss, H.-P., Komora, N., Teixeira, P., & Pintado, M. (2021). The most important attributes of beef sensory quality and production variables that can affect it: A review. *Livestock Science, 250*, Article 104573.
Sarrigiannidis, S. O., Rey, J. M., Dobre, O., González-García, C., Dalby, M. J., & Salmeron-Sanchez, M. (2021). A tough act to follow: Collagen hydrogel modifications to improve mechanical and growth factor loading capabilities. *Materials Today Bio, 10*, Article 100098.
Schüler, K., Marques, D. M., Gusmão, A., Jabouille, M., Leite, M., Cabral, J. M., et al. (2024). 3D printing of plant-based fat inks towards manufacturing complex cellular agriculture products with fatty structures. *Food Hydrocolloids, 110369*.
Sha, L., & Xiong, Y. L. (2020). Plant protein-based alternatives of reconstructed meat: Science, technology, and challenges. *Trends in Food Science & Technology, 102*, 51–61.
Shi, X., Ostrovidov, S., Zhao, Y., Liang, X., Kasuya, M., Kurihara, K., et al. (2015). Microfluidic spinning of cell-responsive grooved microfibers. *Advanced Functional Materials, 25*, 2250–2259.
Shyam, R., & Palaniappan, A. (2023). Effect of sterilization techniques on biomaterial inks' properties and 3D bioprinting parameters. *Bioprinting, e00294*.
Sinke, P. (2021). *LCA of cultivated meat Future projections for different scenarios*.
Skardal, A., Zhang, J., & Prestwich, G. D. (2010). Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates. *Biomaterials, 31*, 6173–6181.
Sogore, T., Guo, M., Sun, N., Jiang, D., Shen, M., & Ding, T. (2024). Microbiological and chemical hazards in cultured meat and methods for their detection. *Comprehensive Reviews in Food Science and Food Safety, 23*, Article e13392.
Somers, S. M., Zhang, N. Y., Morrissette-McAlmon, J. B., Tran, K., Mao, H.-Q., & Grayson, W. L. (2019). Myoblast maturity on aligned microfiber bundles at the onset of strain application impacts myogenic outcomes. *Acta Biomaterialia, 94*, 232–242.
Stout, A. J., Mirliani, A. B., Soule-Albridge, E. L., Cohen, J. M., & Kaplan, D. L. (2020). Engineering carotenoid production in mammalian cells for nutritionally enhanced cell-cultured foods. *Metabolic Engineering, 62*, 126–137.
Su, R., Ai, Y., Wang, J., Wu, L., Sun, H., Ding, M., et al. (2024). Engineered microfibers for tissue engineering. *ACS Applied Bio Materials, 7*, 5823–5840.
Su, L., Jing, L., Zeng, X., Chen, T., Liu, H., Kong, Y., et al. (2023). 3D-Printed prolamin scaffolds for cell-based meat culture. *Advanced Materials, 35*, Article 2207397.
Sun, K., Li, X., & Scherer, P. E. (2023). Extracellular matrix (ECM) and fibrosis in adipose tissue: Overview and perspectives. *Comprehensive Physiology, 13*, 4387.
Sun, W., Tang, H., Wang, L., Du, G., Zhou, J., Chen, J., et al. (2024). Rational scaling-up strategy for cultured meat production based on bioreactor micro-environment evaluation. *Food Bioscience*, Article 105737.
Sun, X., & Zemel, M. B. (2009). Leucine modulation of mitochondrial mass and oxygen consumption in skeletal muscle cells and adipocytes. *Nutrition & metabolism, 6*, 1–8.
Tan, K. Y., Teo, K. L., Lim, J. F., Chen, A. K., Reuveny, S., & Oh, S. K. (2015). Serum-free media formulations are cell line–specific and require optimization for microcarrier culture. *Cytotherapy, 17*, 1152–1165.
Thangadurai, M., Srinivasan, S. S., Sekar, M. P., Sethuraman, S., & Sundaramurthi, D. (2024). Emerging perspectives on 3D printed bioreactors for clinical translation of engineered and bioprinted tissue constructs. *Journal of Materials Chemistry B, 12*, 350–381.
Tibrewal, K., Dandekar, P., & Jain, R. (2023). Extrusion-based sustainable 3D bioprinting of meat & its analogues: A review. *Bioprinting, 29*, Article e00256.
Todhunter, M. E., Jubair, S., Verma, R., Saqe, R., Shen, K., & Duffy, B. (2024). Artificial intelligence and machine learning applications for cultured meat. *Frontiers in Artificial Intelligence, 7*, Article 1424012.
Tuomisto, H. L., & Teixeira de Mattos, M. J. (2011). Environmental impacts of cultured meat production. *Environmental science & technology, 45*, 6117–6123.
Van Eelen, W., Van Kooten, W., & Westerhof, W. (1999). Industrial scale production of meat from in vitro cell cultures. World Intellectual Property Organisation. In. WO/99/31222, filed December 18, 1997, and issued Patent June 24, 1999.
Venturino, I., Vurro, V., Bonfadini, S., Moschetta, M., Perotto, S., Sesti, V., et al. (2023). Skeletal muscle cells opto-stimulation by intramembrane molecular transducers. *Communications Biology, 6*, 1148.
Wang, M. O., Piard, C. M., Melchiorri, A., Dreher, M. L., & Fisher, J. P. (2015). Evaluating changes in structure and cytotoxicity during in vitro degradation of three-dimensional printed scaffolds. *Tissue Engineering Part A, 21*, 1642–1653.
Wang, X., Wang, M., Xu, Y., Yin, J., & Hu, J. (2024). A 3D-printable gelatin/alginate/ε-poly-l-lysine hydrogel scaffold to enable porcine muscle stem cells expansion and differentiation for cultured meat development. *International Journal of Biological Macromolecules*, Article 131980.
Wei, Z., Dai, S., Huang, J., Hu, X., Ge, C., Zhang, X., et al. (2023). Soy protein amyloid fibril scaffold for cultivated meat application. *ACS applied materials & interfaces, 15*, 15108–15119.
Xiang, N., Yuen Jr, J. S., Stout, A. J., Rubio, N. R., Chen, Y., & Kaplan, D. L. (2022). 3D porous scaffolds from wheat glutenin for cultured meat applications. *Biomaterials, 285*, Article 121543.
Yang, R., Fei, Z., Wang, L., Tang, H., Sun, W., Li, M., et al. (2024). Highly efficient isolation and 3D printing of fibroblasts for cultured meat production. *Frontiers in Sustainable Food Systems, 8*, Article 1358862.
Yeo, M., Lee, H., & Kim, G. H. (2016). Combining a micro/nano-hierarchical scaffold with cell-printing of myoblasts induces cell alignment and differentiation favorable to skeletal muscle tissue regeneration. *Biofabrication, 8*, Article 035021.
Yerrabelli, R. S., Somers, S. M., Grayson, W. L., & Spector, A. A. (2021). Modeling the mechanics of fibrous-porous scaffolds for skeletal muscle regeneration. *Medical, & Biological Engineering & Computing, 59*, 131–142.
Yuan, Y., Zhang, H., Li, Y.-F., Xue, X.-N., Zhang, G.-H., & Zhang, H. (2024). Mechanism of static loading injury in human skeletal muscle cells. *American Journal of Translational Research, 16*, 1135.
Zheng, Y.-Y., Hu, Z.-N., & Zhou, G.-H. (2024). A review: Analysis of technical challenges in cultured meat production and its commercialization. *Critical Reviews in Food Science and Nutrition*, 1–18.
Zhuge, W., Ding, X., Zhang, W., Zhang, D., Wang, H., & Wang, J. (2022). Microfluidic generation of helical micromotors for muscle tissue engineering. *Chemical Engineering Journal, 447*, Article 137455.
Zidarič, T., Milojević, M., Vajda, J., Vihar, B., & Maver, U. (2020). Cultured meat: Meat industry hand in hand with biomedical production methods. *Food Engineering Reviews, 12*, 498–519.
Zoda, A., Kagawa, R., Obinata, R., Urakawa, M., Oono, Y., Ogawa, S., et al. (2022). Genetic relationship between superovulatory response traits and carcass traits in Japanese Black cattle. *Animal Science Journal, 93*, Article e13731.