Technology and processes for cultivated meat

Fundamentals, challenges, and perspectives

Marie-Luise Schlieker*, Laurenz Köhne*, Katharina Julia Brenner, Carina Theresa Linnemann, Marius Henkel

Abstract

Cultivated meat, along with plant-based and insect-based alternatives, as well as proteins and biomass derived from fermentation processes, is considered a potentially sustainable alternative to conventional meat production. This review provides an overview of the concept of cultivated meat production and explains what efforts are currently being made in research and development to establish it as a sustainable and healthy food. The focus is on the current (bio)technological challenges, especially in production. To produce a structured product that resembles animal tissue, the growth of cells on suitable edible and biocompatible scaffold matrices is a prerequisite. In addition to this approach, there are also non-scaffold based methods such as 3D-bioprinting. Regardless of the method chosen, however, scaling up the growth and differentiation processes is crucial to increase the efficiency of the entire process. This article provides an overview of the current state of the art and derives approaches for future research projects, taking sustainability aspects into account.

Citation
Schlieker M-L, Köhne L, Brenner KJ, Linnemann CT, Henkel M: Technology and processes for cultivated meat. Fundamentals, challenges, and perspectives. Ernahrungs Umschau 2025; 72(6): 114–21.e30–1.

Open access
This article is available online: DOI: 10.4455/eu.2025.025

Peer reviewed
Manuscript (overview) submitted: 4 September 2024; revision accepted: 9 January 2025

Marie-Luise Schlieker, M.Sc.[1, 2]
Laurenz Köhne, M.Sc.[1]
Katharina Julia Brenner, M.Sc.[1]
Carina Theresa Linnemann, M.Sc.[1]
Prof. Dr.-Ing. Marius Henkel[1, 2]
[1] Cellular Agriculture, TUM School of Life Sciences, Technical University of Munich
Gregor-Mendel-Strasse 4, 85354 Freising, Germany
[2] Munich Institute of Integrated Materials, Energy and Process Engineering (MEP), Technicl University of Munich
Lichtenbergstraße 4a, 85748 Garching, Germany

Introduction

In the European Union, industrial livestock farming accounts for around 85% of total agricultural CO_2 emissions [1]. In contrast to conventional agriculture, cultivated meat requires significantly less agricultural land, which is particularly important given limited resources [2]. It addresses the fundamental conflict of objectives between food security, environmental preservation, and health, known as the diet-environment-health trilemma. Cultivated meat has the potential to positively influence all three aspects of this trilemma at the same time [2, 3]. The term refers generally to structured tissue produced from animal cells using modern cell cultivation methods. The general approach is to circumvent the ethical and ecological problems of traditional livestock farming without consumers having to forego the consumption of animal proteins and fats [4]. This modern method could fundamentally change the food industry and provide more sustainable and ethical alternatives to conventional meat consumption. Cultivated meat could not only significantly reduce the environmental impact of livestock farming and address the diet-environment-health trilemma but also solve the global problem of animal suffering. In addition, consistent and safe meat production can be ensured by growing animal muscle and fat cells in controlled environments. There is also ongoing research into whether production requires fewer resources while minimizing CO_2 emissions compared to conventional livestock farming.

* contributed equally

Nutritional and physiological aspects of (cultivated) meat

Personal health considerations are consistently identified in surveys as one of the primary drivers behind the adoption of a vegetarian diet [5]. Despite a continuous decline in average meat consumption in Germany since 2019, current intake remains at 1 kg/week, exceeding the recommendation of the German Nutrition Society (*Deutsche Gesellschaft für Ernährung*, DGE) by more than threefold [6]. In February 2024, the DGE revised its evidence-based dietary guidelines, halving the recommended weekly consumption of meat and processed meat products to 300 g [7]. According to the National Nutrition Survey II, the intake of vitamin D, folic acid, iodine, iron, calcium, long-chain polyunsaturated fatty acids (LC-PUFA) and fiber is often not covered in Germany [8]. A vegetarian diet is theoretically possible in all age groups to cover requirements [9]. However, it requires targeted planning, especially with regard to critical nutrients such as vitamin B_{12}, iron, zinc, iodine and long-chain n-3 fatty acids, the intake of which must be monitored to prevent deficiencies [10]. In practice, however, this monitoring is often a challenge, as both knowledge and practical implementation are often lacking. Animal proteins are of particularly high quality as they have a complete amino acid pattern and high bioavailability [11]. In addition, meat products provide essential nutrients such as iron, zinc, selenium, vitamin B_{12} and other vitamins in forms that can be efficiently absorbed by the human body, which can reduce the risk of malnutrition. However, the nutritional disadvantages of increased meat consumption mainly stem from low fiber content and the high intake of saturated fatty acids and n-6 fatty acids [8, 12]. In addition, the development of cultivated meat could help to reduce conventional factory farming, which is associated with an increased risk of zoonoses [13] and the development of antibiotic resistance due to the intensive use of antibiotics [14]. As the processes involved in the production, maturation and packaging of cultivated meat have hardly been studied to date, it is particularly important to minimize potential risks. Performing these steps in a sterile manner could help to significantly reduce the risk of contamination and harmful meat spoilage. In the development of cultivated meat, the fundamental concept of preserving the positive nutritional aspects of meat while avoiding or reducing the negative aspects is therefore of particular importance.

Structure of meat

In order to create a structured product that resembles animal tissue, it is necessary to take into account the structure of natural muscle tissue. The fiber-based structure of meat is the result of a complex hierarchical tissue structure, which is chemically composed of 75% water, 20% protein, 1–10% fat and 1% glycogen. The most important functional unit is the muscle fiber, also called myofiber or muscle cell, which is surrounded by connective tissue, intramuscular fat, blood vessels and nerves. Muscle fibers, fat and connective tissue are the main determinants of muscle condition and quality. The muscle fibers are organized in bundles called fascicles. These bundles contain one to two thousand myofibrils, the subunits of which are myofilaments. The myofilament consists mainly of myosin components, which are responsible for muscle contraction (◆ Figure 1) [15]. The muscle cells are embedded in dense and complexly organized connective tissue, the so-called extracellular matrix (ECM). In order to achieve the texture of cultivated meat, the mechanical properties of the ECM must be reproduced [16].

Production of cultivated meat

Broadly, the cultivated meat production process can be divided into four main areas: (1) obtaining the cells, (2) multiplying the cells, (3) differentiating the cells into skeletal muscle cells and fibers as well as (4) packaging and maturation into the final meat product (◆ Figure 2) [17]. Various cell types can be used for cultivated meat, with muscle, fat and connective tissue cells playing the most important role. For the production of cultivated meat, the starting cell types must be able to renew themselves in sufficient quantities (proliferation) and subsequently differentiate into mature cells, which form the basis for the three-dimensional structure of animal tissue. The most commonly used method for obtaining the required cells is to perform an invasive biopsy on a living animal [18]. A biopsy can be used to obtain so-called primary cells, such as satellite cells (muscle stem cells). Natural cells obtained in this way can only divide a limited number of times [18]. Current research is focusing on the creation of suitable cell lines with unlimited division capacity, so-called immortalized cells, in order to address ethical issues of continuous biopsy interventions on living animals and to positively influence process efficiency through longer process runtimes [19]. A promising, ethically safe source of stem cells is also umbilical cord blood, which is obtained without animal suffering. One advantage of these primary cells is that they can be isolated directly from the tissue and used without the use of cell lines. This avoids possible disadvantages such as genetic changes, complex culture requirements and limited physiological similarity to natural cells. The hematopoietic stem cells (HSCs) contained in umbilical cord blood primarily form blood cells and are therefore not directly suitable for the production of muscle or fat cells. Mesenchymal stem cells (MSCs) from umbilical cord blood, on the other hand, could re-

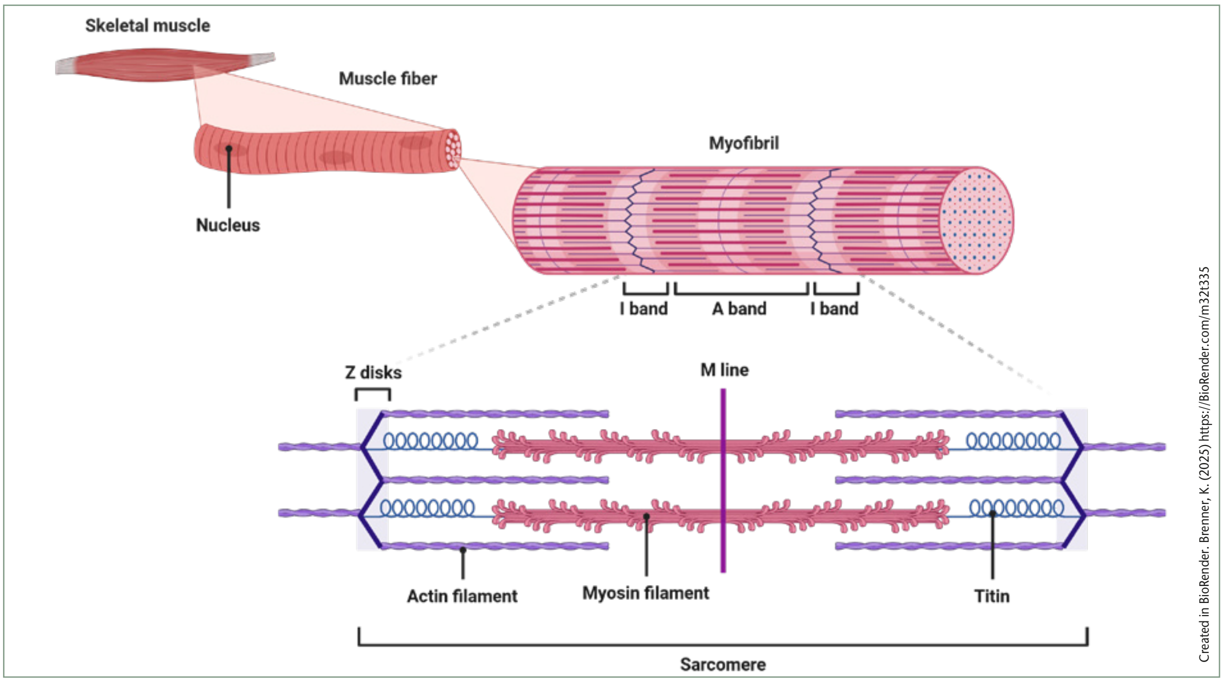

Fig. 1: **Illustration of muscle architecture** (own presentation)
A skeletal muscle consists mainly of muscle fibers. These, in turn, consist of myofibrils. The filaments contain parallel protein filaments of myosin and actin.

present a potential basis for cultivated meat due to their ability to differentiate into muscle, fat and connective tissue cells. However, the amount of MSCs in umbilical cord blood is limited [20], which is why other sources such as induced pluripotent stem cells (iPSCs) are often preferred. After collection, the desired cells are isolated and transferred to a culture medium. An important component of the medium for efficient cell growth is fetal calf serum (FCS), which contains amino acids, proteins, growth factors, hormones, vitamins and trace elements [21]. The use of FCS for cell growth has its origins in medical research and development. By using FCS, it was possible to meet the complex requirements of cells in the laboratory and significantly advance medical developments from which we benefit today. However, the use of FCS is not compatible with the ethical requirements of cultivated meat [22], nor is it a usable basis for large-scale production. For this reason, numerous research projects are focusing on the development of cost-effective and FCS-free media for cell culture, often based on plant ingredients [23]. Various methods can be used to propagate cells. The most commonly used technique is the cultivation of cells in single layers (monolayer), which is mostly used for research purposes. Most cell types, including those found in conventional and cultivated meat, require anchoring in order to adhere, proliferate and differentiate [24]. Two-dimensional (2D) monolayer cultures allow cells to access a nutrient-rich culture medium while interacting with neighboring cells and a rigid structure at the bottom that provides a surface for integrins to attach [25]. Integrins are transmembrane proteins that play a crucial role in cell-cell and cell-matrix interactions. However, the cells present in organs require not only an anchorage for proliferation, but also a three-dimensional (3D) matrix with specific biochemical and mechanical properties. This environment is formed by the ECM, which consists of different components and has a characteristic organizational pattern that is absent in 2D cultures [24].

Methods for producing meat with an authentic texture

Providing an ECM environment that mimics the required conditions of animal tissues is one of the key challenges for the production of cultivated meat. The 3D scaffold structures used in this process are known as bioscaffolds. They serve to attach and multiply animal cells and offer the possibility of creating a spatial arrangement of the differentiated tissue structures (including muscle vs. fat) [26]. One of the greatest challenges here is the supply of nutrients and oxygen to the tissue.
This supply of nutrients based on diffusion is only possible efficiently to a depth of less than half a millimeter [27]. In mammalian tissue, diffusion distances of 10–30 μm can typically be observed. In fact, the maximum distance between a cell and its nearest capillary is rarely greater than 200 μm and is usually less than 100 μm [28]. To ensure an efficient supply of nutrients during tissue cell

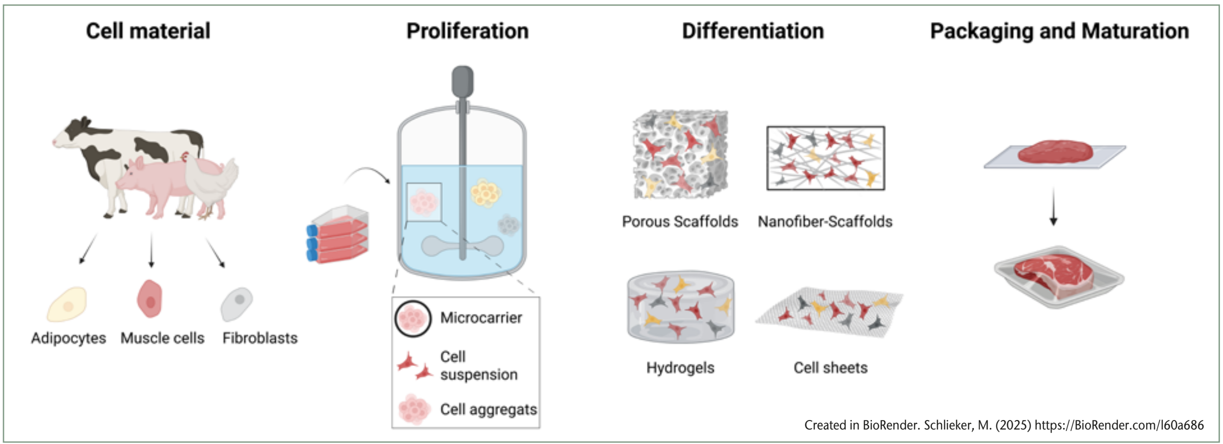

Fig. 2: **Schematic representation of the general process for the production of cultivated meat** (own presentation)
A: isolation of cells and initial proliferation; B: large-scale cell expansion; C: differentiation of cells into skeletal muscle cells and fibers; D: packaging and maturation into the final meat product

culture, the scaffold structures must allow additional mass transport via convection (channels for nutrient supply) or an increase in surface area (porosity) [24]. There are different types of scaffolds with versatile 3D structures, including microcarriers, porous matrices, nanofibers, hydrogels and cell sheet technology [17].

Microcarrier systems consist of small spherical beads (microcarriers) that provide a surface for cell attachment, whereby the cells form either aggregates or cell-microcarrier complexes. Thus, a multiple of the surface area of 2D cultures can be achieved. The microcarriers are suspended in the culture medium of a bioreactor, simultaneously providing a 3D culture environment and a 2D surface for cell growth. This technique provides a large surface area for cell growth and supports cell differentiation. However, the currently established carrier materials are optimized for efficient use in pharmaceutical production processes and are generally not suitable for human consumption [29].

Three-dimensional, porous scaffolds made of polymers can simplify fundamental challenges in the production of cultivated meat by supporting cell growth, tissue regeneration and the provision of bioactive compounds. Their high porosity enables efficient nutrient and oxygen transport, creating an optimal environment for cell adhesion and differentiation. These scaffolds resemble natural ECM and support the integration and functionality of cell and basement membranes. These scaffolds effectively support the growth of animal cells and resemble real meat in texture and color [17].

Nanofiber scaffolds can also mimic the natural ECM and promote cell adhesion, proliferation and differentiation. Various approaches such as electrospinning are used to produce these scaffolds [17]. Despite their advantages, the complexity of manufacturing for food production and the challenges of scaling up electrospinning processes remain limitations [30].

Hydrogels are polymers with a high water absorption capacity that provide mechanical support for cell growth and are similar to natural ECM. Despite their potential, challenges such as suitable mechanical properties, biocompatibility and uniform nutrient distribution, especially from the nutrient-rich medium to the interior of the hydrogels, need to be overcome [31].

In addition to the technologies described here, which utilize the fundamental advantages of bioscaffolds, research is also investigating so-called cell sheet technology. This does not require 3D scaffolds and could potentially be more cost-effective and scalable. Cell sheet technology allows the cultivation and detachment of cells on temperature- or pH-sensitive surfaces by specifically adapting the environmental conditions, whereby the cell-cell connections and the ECM are preserved and a gentle transfer to target structures is made possible [32]. The layer-by-layer assembly allows control of the spatial distribution of cells, growth factors and other molecules within the scaffold while mimicking the hierarchical organization found in native tissues [33]. Compared to conventional methods, the challenges lie in the control of tissue structure and consistency [17].

In the processing phase of the final product, one goal is to process the cultivated tissue into a product that is indistinguishable from conventional meat products in terms of taste and appearance [34]. In particular, traditional processes for maturing freshly slaughtered raw meat can be used here. The controlled storage of raw meat has a positive effect on structure and taste, for example by releasing enzymes. It remains to be investigated, to what extent these processes can be implemented for cultivated meat. In the future, the tissues produced could also be further refined to produce special products such as burgers, sausages, or steaks. In order to enable a controlled composition, various studies are being carried out to determine whether media residues and residues such as growth factors can be removed during the production process. Health aspects as well as consumer acceptance and the simplification of a future approval process play a role here [17].

Overview of bioreactor types for cultivated meat production

In principle, a variety of different bioreactors can be used for the production of cultivated meat (◆ Figure 3). Many of these bioreactors are already used in industrial biotechnology. The type of bioreactor used and its design must be carefully selected depending on the cells used, bioscaffold materials and planned operating mode. Roughly speaking, bioreactors can be divided into two different groups. The first group includes bioreactors in which the cells can grow adherently on a surface, while in the other group, the individual cells are in suspension and can form aggregates there, among other things. Furthermore, the reactors can be operated in three different ways. One method is the batch method, in which all the required components are added to the reactor in advance and the process is terminated once the nutrients in the medium have been used up. A further development of the batch process is the fed-batch process. Here, new medium is continuously added during cultivation without removing used medium. Another option is the perfusion process, in which continuous medium is added and then removed [35, 36]. In contrast to fed-batch, this method enables exchange of the used medium (various bioreactor systems see ◆ Box).

Overall, bioreactors that are specifically designed for use with suspension cultures [36] can be distinguished from bioreactors for adherent growth (◆ Figure 3). The former are particularly suitable for the production of meat products that do not require a defined structure, such as minced meat, nuggets or for processes in which the proliferation and differentiation of cells can be separated from each other. However, perfusion processes can also be used to enable more complex structures in cultivated meat. These usually ensure slower growth that is limited or predetermined by the structure of the scaffold [44].

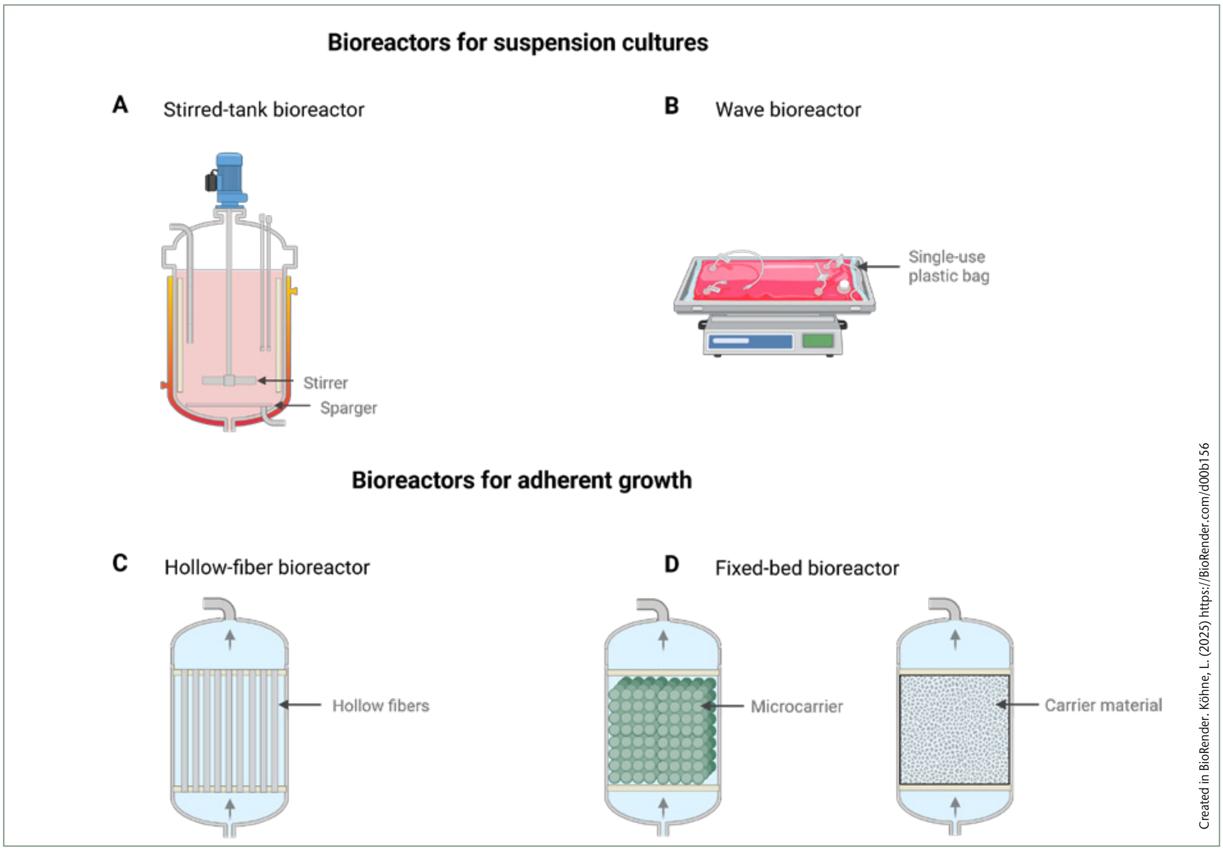

Fig. 3: **Schematic overview of different bioreactor systems** (own presentation)
A: stirred-tank bioreactor; B: wave bioreactor; C: hollow-fiber bioreactor; D: fixed-bed bioreactor

Stirred-tank bioreactors

Stirred-tank reactor (STR) is the most widely used type of reactor in industrial biotechnology. It is a vessel with an integrated agitator, which ensures mixing and dispersion of the gas phase into small bubbles in the reactor and thus more efficient oxygen transfer [37, 38]. When cultivating animal cells, however, it should be noted that, depending on the agitator used and the power input, the shear stress for the cells can be too high [39]. In principle, cell culture can be cultivated in a relatively large volume using a stirred tank bioreactor. However, this type of reactor requires that the culture is either in suspension or that micro-carriers are used as carrier materials. The stirred-tank bioreactor is therefore more suitable for unstructured products, i.e. a process that focuses on cell [36].

Fixed-bed bioreactors

In this type of bioreactor, medium is pumped through a solid carrier material (fixed bed) (◆ Figure 3), on which cells are immobilized [37]. In order to make this functional principle usable for cultivated meat, carrier materials must be used that are biocompatible and edible, or the carrier material must be separated from the cells and be able to be removed afterwards [40]. Usually, the fixed carriers, together with the adherent cells, stay in the same place. In many cases, this enables better control over the three-dimensional structure, but it can lead to the formation of pronounced diffusion gradients [36], which makes nutrient supply more difficult.

The characteristic limitations of fixed-bed reactors described here can be largely eliminated, for example, by using fluidized-bed bioreactors, in which the carrier material is kept in a fluidized state by an upward media stream.

Hollow-fiber bioreactors (membrane bioreactors)

The hollow-fiber bioreactor typically consists of thin hollow fibers or usually cylindrical hollow membranes to which the cells can attach efficiently. The cells can thus grow on the outside of the membrane and are supplied with medium from the inside through the membrane [37]. This and the lower shear forces make this type of reactor well suited for perfusion processes. A nutrient gradient can occur on a larger scale or with longer systems. This means that the cells may be undersupplied as the bioreactor progresses, depending on the process control. It should also be noted that the diameter of the hollow membranes has an important influence on the reactor. With a larger diameter, efficiency decreases as there is less surface area available for the cells. However, if the diameter is smaller, the pressure required for efficient flushing increases, which can damage the membranes and cells. For this reason, it may make more sense to use parallelized reactors ("numbering-up") than to scale them up in size ("scaling-up") [41].

Wave bioreactors

Wave bioreactors are used as single-use bioreactors. A major advantage is that the reactors are pre-sterilized and therefore do not require time-consuming cleaning after each process [36]. However, larger quantities of plastic are produced here, which subsequently represents a major environmental problem. Not all single-use bioreactors are wave bioreactors. There are also single-use stirred-tank bioreactors and other types of single-use bioreactors that can be used for cell culture [42]. The functionality of wave bioreactors is based on a tilting movement that sets the medium in motion and mixes it. However, these single-use bioreactors are limited in size, particularly with regard to the mechanical forces generated by the tilting movement. In addition, support structures must be provided for larger volumes in order to protect the bag from damage [42, 43]. A core limitation when using this technology is the lower oxygen transfer, as this only takes place via the surface of the media. In addition, mixing is severely restricted due to the low shear forces. However, this is not usually considered a relevant disadvantage for cell culture in practice due to existing kinetics.

A major disadvantage of perfusion methods is the high consumption of culture medium, which is one of the biggest cost factors in the production of cultivated meat [41]. In order to improve this in terms of sustainability, the possibility of recycling medium should always be investigated when developing processes for the efficient production of cultivated meat [45]. Medium that has not been completely used up should be reprocessed, i.e. missing nutrients should be added and metabolic interfering products removed. There are also already approaches to use the spent medium for the cultivation of other organisms, such as algae. The algae cultivated in this way can, for example, then be added to the medium again in order to return nutrients [44, 46].

The NFS project "PERFEG-MEAT" aims to apply two strategies that enable more efficient use of growth factors. Due to their limited availability, these factors can currently not be supplied in the quantities required for cultivated meat production. In addition, the production of nature-identical growth factors is still expensive and their use requires significant optimization due to their short half-lives. To address these challenges without genetically modifying the cells themselves, the following options are available: The first option for a more efficient use of growth factors is the use of recycling step for perfusion processes to reduce the high demand for media components. By increasing the retention time of the medium, the availability of unused nutrient components for the cells is extended. However, a balance must be found between the prolonged use of the medium and the accumulation of inhibitory metabolic products of the cells or these must be specifically removed, for example by dialysis processes. The second approach deals with the development and use of modified or artificial growth factors, which enable more efficient processes due to their properties.

Conclusion

Cultivated meat has great potential to supplement or even replace conventional animal production and thus change economic, social and ecological habits [47]. In the future, controlled process conditions should reduce resource consumption for the production of an alternative meat product. Overall, the progress made so far shows that the foundations have been laid for the establishment of cultivated meat. Nevertheless, extensive challenges remain, such as the high production costs and the lack of clarity as to how scaling up to an industrial scale can be achieved. Until now, no process has been identified in which cultivated meat can be produced economically and sustainably. Future developments must also focus on the sustainability and safety of the product, as these factors will play a crucial role in the conception and design of industrial plants, consumer acceptance and a future approval procedure in the EU [45]. Particular attention should be paid to the following areas: research, regulatory framework, consumer education, interdisciplinary approaches and sustainability indicators. In biotechnological research, key topics such as the optimization of the cell culture medium, bioreactor design, and cell line development must be further advanced. In addition, the creation of a clear regulatory framework within the EU is essential, particularly with regard to consumer acceptance. Such a framework, which focuses on the safety of the end product and the processes for producing cultivated meat, could strengthen consumer confidence. At the same time, specific sustainability criteria should be defined to make the environmental impact of cultivated meat more comparable with existing production methods. The further development of cultivated meat also requires interdisciplinary cooperation between biotechnologists, engineers, economists and social scientists in order to successfully solve both technical and social challenges. In today's world, it is our responsibility to help shape the future of animal products through research and development in the field of cellular agriculture. Current efforts are aimed at developing and establishing more sustainable and ethical alternatives to conventional animal products. By laying the foundations for innovative approaches today, we are laying the foundations for the food of the future.

Funding
This work emerged from "Innovationsraum NewFoodSystems" (Project PERFEG-MEAT) and was funded by the Federal Ministry of Research, Technology and Space (BMFTR) as part of the "Innovationsräume Bioökonomie" and "Nationalen Forschungsstrategie BioÖkonomie 2030". Funding reference: 031B1480A.

NewFoodSystems
Neue Lebensmittelsysteme
Gefördert durch:
Bundesministerium für Forschung, Technologie und Raumfahrt

Acknowledgements
This work was supported by the TUM WACKER Institute for Industrial Biotechnology. The authors would like to thank Dr. Carsten Bornhövd and Dr. Laura Lindenthal (Wacker Chemie AG) for their support and constructive discussions as part of the "ArtMeat" project of the TUM WACKER Institute for Industrial Biotechnology.
For more information on the *Innovationsraum* NewFoodSystems, visit → www.newfoodsystems.de

Disclosures on Conflicts of Interest and the use of AI
The authors declare that there is no conflict of interest. AI was used for language optimization and to create/check translations.