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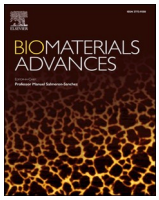

# Recent advances in scaffolding biomaterials for cultivated meat

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ABSTRACT

The emergence of cultivated meat provides a sustainable and ethical alternative to traditional animal agriculture, highlighting its increasing importance in the food industry. Biomaterial scaffolds are critical components in cultivated meat production for enabling cell adhesion, proliferation, differentiation, and orientation. While there's extensive research on scaffolding biomaterials, applying them to cultivated meat production poses distinct challenges, with each material offering its own set of advantages and disadvantages. This review summarizes the most recent scaffolding biomaterials used in the last five years for cell-cultured meat, detailing their respective advantages and disadvantages. We suggest future research directions and provide recommendations for scaffolds that support scalable, cost-effective, and safe high-quality meat production. Additionally, we highlight commercial challenges cultivated meat faces, encompassing bioreactor design, cell culture mediums, and regulatory and food safety issues. In summary, this review provides a comprehensive guide and valuable insights for researchers and companies in the field of cultivated meat production.

## 1. Introduction

Cellular agriculture is an emerging field focusing on the production of agricultural products from cell cultures rather than whole plants or animals [1,2]. Compared to traditional agriculture, cellular agriculture can be used to produce a variety of products, including meat, dairy, and other animal products, without the need for traditional livestock farming [3,4]. By utilizing cell cultures, cellular agriculture seeks to address some significant environmental, ethical, and public health issues associated with conventional animal agriculture. For example, it has been reported that >75 % of infectious diseases in humans stem from animal sources due to the increased close human-animal contact from animal agriculture, the destruction of wildlife habitats, and the increasing human population and global mobility [5]. Moreover, the use of antibiotic resistance in intensive animal production contributes significantly to the development and spread of antibiotic resistance in animals and food of animal origin [6]. Intensive animal agriculture also contributes to climate change due to land and waste usage [7]. This is primarily due to the production of animal feed and contamination from animal waste. According to the 2022 UN Environment Programme report on addressing the food and climate change issue, intensive protein production is intimately linked with anthropogenic stressors, including land use change, biodiversity decline, and environmental pollution, which eventually cause zoonotic diseases [8]. Although public concerns about climate change appear to drive purchasing behavior changes in beef consumption to some extent, global meat consumption continues to rise steadily [9,10]. Despite the increasing awareness of climate change, this trend persists, indicating that the aspiration to mitigate climate impact alone is insufficient to significantly reduce meat consumption. Thus, a more practical approach to addressing the issues caused by meat production lies in altering the production process itself, rather than extensive consumer behavior change. Thus, cellular agriculture presents a revolutionary solution for meat production by embracing the idea of cell cultivated or lab-grown meat.

Cultivated meat, also known as cell-based or cell-cultured meat, is produced by culturing animal stem cells to mimic the organoleptic and nutritional properties of conventional meat [11–14]. The world's first cultured beef burger, which was made from bovine stem cells, was produced by Dr. Mark Post's team in 2013 [15]. Recently, Israel's startup, Aleph Farms, unveiled 'World's First' Lab-Grown, slaughter-free steak and submitted its application for approval to Food Standard Agency (FSA) in the United Kingdom [16]. In 2023, two US companies, Upside Foods and Good Meat, announced that they received approval

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from the US Department of Agriculture to start selling their cultivated chicken [17,18]. Unlike conventional agriculture, this cultivated chicken was grown in bioreactors in an urban factory in California. Over the past decade, there has been a significant increase in the number of companies aiming to commercialize and scale their cell-cultivated meat production.

The production process of cultivated meat comprises several key steps, starting with cell selections [19]. Various types of stem cells can be isolated from an animal through a biopsy or non-invasive approaches [20–24]. These cells are then cultured in bioreactors using nutrition-rich culture medium that simulates the natural environment, where they proliferate and differentiate into different types of muscle cells. Typically, the medium, optimized to support cell growth, is contained within a bioreactor that maintains ideal conditions of temperature, pH, and oxygen conditions [25]. A critical step during cultivated meat production is the seeding of cells onto a 3D scaffold. The scaffold often plays a crucial role for efficient transportation of oxygen, nutrients, and waste products to and from the cells, maintaining the growing tissue's morphology, and providing structural support to the final product. In addition, in order to enhance cell growth, proliferation, and 3D tissue development, biomechanical and biochemical cues can be employed to ensure the texture and nutrition content are comparable to traditionally harvested meat. These cues are governed by the biochemical and mechanical characteristics of the surrounding extracellular matrix (ECM), which supports 3D tissue growth in the development of a structural, functional tissue product similar to traditional meat [26]. Scaffolds serve as the architectural foundations for cell growth, offering a supportive three-dimensional matrix where cells can adhere, proliferate, and differentiate to form muscle and fat tissues [11,27]. Furthermore, a scaffold should recapitulate the natural 3D microenvironment of the cells, which is crucial for cell functions through cell-cell interactions and cell-matrix interactions. Consequently, culturing cells in a suitable 3D matrix will have an important impact on cell behaviors, potentially leading to more in vivo-like tissue structures and improved organoleptic properties [26]. These scaffolds are designed to mimic the natural extracellular environment, thus fostering the development of cultivated meat with textural and nutritional characteristics that are similar to traditional meat.

The choice of scaffold significantly influences the texture, structure, and overall quality of the resulting cultivated meat product. Thus, in selecting biomaterials for scaffolds, which is also called scaffolding biomaterials, several factors should be considered, including biocompatibility, porosity, and mechanical properties [12,28–30]. The most commonly used materials include natural polymers such as alginate, collagen, chitosan, and fibrin, which offer high compatibility that can closely replicate the natural environment of tissue growth and proliferate. Additionally, synthetic polymers, such as poly(lactic-*co*-glycolic acid) (PLGA) and polyethylene glycol (PEG), are being investigated for their tunable properties and degradability. Over the last decade, by incorporating advanced biomanufacturing techniques like 3D bioprinting with scaffolding biomaterials, researchers are able to precisely control cell distribution and tissue architectures for 3D cultivated meat production [31–33]. In addition, innovative approaches, such as the use of edible and biodegradable materials, and the development of scaffold-free systems, are also being investigated [34–38].

Despite the advancements in scaffolding materials, the challenges of 3D tissue culture must be addressed in a scalable, cost-effective, and food-safe manner. Although the technology remains in its early stages, significant progress has been made in scaling up production and reducing costs. Researchers are continually refining the process to improve the texture, taste, and nutritional profile of the cultivated meat, with the goal of offering a viable and desirable alternative to conventionally produced meat. In this review, we focus on the different biomaterials that are utilized as scaffolds for the application of cultivated meat, drawing on literature from cultivated meat and adjacent disciplines such as biomedical tissue engineering. Specifically, in Section 2, we introduced the criteria of cultivated meat production and outlined the general production process, which includes cell isolation, expansion, tissue maturation, and food product processing. In Section 3, we highlighted the recent studies (from the last five years) on scaffolding strategies, such as decellularization, microcarriers, porous scaffolds, nanofibrous scaffolds, hydrogel, and scaffold-free technologies. In Section 4, we focused on different types of biomaterials, such as natural biomaterials, synthetic biomaterials, plant-based edible polymers, and self-assembling peptides. We then explore the challenges and future prospects of cultivated meat, encompassing topics like cell culture mediums, bioreactors, and considerations for regulatory and food safety. Overall, this review will provide valuable insight for the researchers and companies in the field of cultivated meat through an in-depth analysis of current scaffolding biomaterials, their advantages and disadvantages, and a comprehensive understanding of the challenges and solutions related to commercialization, scalability, and regulatory compliance.

## 2. Cultivated meat

Despite significant advancements made in cultivated meat by both academic research groups and commercial companies, it remains a relatively nascent research field due to its challenges in scalability and cost. In this section, we summarize the key considerations for choosing scaffolding biomaterials for cultivated meat and outline the general production process.

### 2.1. Criteria for cultivated meat production

The production of cultivated meat adheres to a set of crucial criterial to ensure viability, safety, and consumer acceptance in the market. The selection of appropriate biomaterials is also critical for cultivated meat production. Biomaterials used for this should be edible, biodegradable, biocompatible, and possess mechanical properties matching the texture and structure of traditional animal meat. In addition, these biomaterials should be economically viable and available at large scale. Importantly, the biomaterials used should allow robust muscle cell growth into tissues with suitable meat texture. In this section, our primary focus is on the criteria for selecting functional biomaterials for cultivated meat production. A fundamental criteria is the biomechanical properties of the cultivated meat to ensure it mimics the textures and structural integrity inherent to traditional meat [39]. In addition, optimal functionality and biocompatibility of the biomaterials are essential, which requires the materials' ability to support cellular adhesion, proliferation, and differentiation to form tissue structures that mimic conventional meat [40]. Moreover, biosafety and biosecurity are two challenges when distributing cultivated meat. Without access to industrial labs, how to keep cultivated meat fresh without the usage of antibiotics is challenging [41]. The inclusion of antibiotics in tissue culture mediums could inevitably lead to the emergence of antibiotic-resistant pathogens [42]. This might risk producing diseases more resistant to our available treatments, making them costlier and harder to address [25,43,44]. Additionally, ensuring that the sensorial and nutritional profile aligns with, or exceeds, that of conventional meat is essential to confirm the health benefits of the cultivated meat [30,45,46]. If cultivated meat is to be consumed, its sensory (texture, color, flavor) and nutritional characteristics are of utmost importance. The sensory properties include molecular characteristics of the product, such as content and nature of proteins, the presence of myoglobin, and the composition of volatile compounds [46]. Alongside sensorial characteristics, the nutritional quality of cultivated meat should closely mimic that of traditional meat. Traditional meat is a nutritionally dense food, rich in high-quality proteins, vitamins, minerals, and other vital nutrients. Many compounds found in the muscle come from animal feed components that have been digested and then altered by organs outside the muscle. If these compounds aren't intentionally introduced into the culture medium and assimilated by the cells, they won't be present in cultivated meat,

impacting its flavor, texture, color, and nutritional value [47]. From a nutritional standpoint, fat in meat can be characterized by its percentage content and fatty acid composition. These characteristics are influenced by factors such as livestock species and breed, age, type of feed, and meat cut.

Moreover, economic viability and the scalability of production processes are critical for the market penetration and sustainability of cultivated meat. It's essential that it can be produced at both a competitive price point and in sufficient quantities. Furthermore, consumer acceptability, dependent on sensory attributes and public perception, must be thoroughly addressed. Transparent communication and education about the production and advantages of cultivated meat are also essential to promote consumer adoption and ensure market success. Lastly, regulatory compliance, which involves meeting international food safety and labeling standards, is crucial to guarantee legal and safety adherence, paving the way for a smooth transition from the lab to the market.

### 2.2. General production process

There are four main phases of the general production of cultivated meat, including cell isolation, cell expansion, scaffold and cell maturation, and final product processing [20,48], Fig. 1. Cell isolation is the first step in the cultivated meat production process. In this phase, specific cells that are capable of making muscle fibers, typically muscle stem cells or satellite cells, are extracted from a live animal, often via biopsy, Fig. 1A. Typical sustainable cell sources include smooth muscle cell, fat cell, and mesenchymal stem cells (MSCs), that can be isolated from cattle, sheep, and chickens [25,49,50]. Several research groups have utilized bovine satellite cells (BSCs), which are dedicated muscle progenitors. However, these cells predominately differentiate into muscle fibers. In contrast, meat is a complex entity, which comprises multiple tissue types such as muscle, fat, and connective tissues [51]. Consequently, it is not sufficient to only use satellite cells for replicating the entire composition of meat, as it needs to incorporate additional cell types, i.e., fat, from other sources. Recently, it has been reported that MSCs is an ideal cell source for producing both primary cell types required for cultivated meat due to their capability of differentiating into both adipogenic (fat) and myogenic (muscle) lineages [52–54]. Furthermore, MSCs can be conveniently isolated from a diverse range of tissues, including bone marrow, adipose tissue, umbilical cord, placenta, and fetal fluids [25,55,56].

The second phase of the production process is cell expansion, where the initially isolated cells are multiplied to achieve large quantities that are viable for meat production, Fig. 1B. Typically, this multiplication process occurs in a bioreactor, where the cells are cultured with a nutrient-dense medium composed of essential amino acids, sugars, and growth factors that promote cell division. The culmination of this phase is a substantial biomass of cells, ready for differentiation into muscle and fat cells. In this step, it is essential to have cost-effective, food-safe medium that support a high rate of proliferation [20,25,57,58]. This is a critical step to maintain a high quantity of cells for the generation of meat tissue. Currently, the most popular approach for cell expansion is using stirred-tank bioreactors [20]. Although the biopharmaceutical industry can culture mammalian cells in stirred bioreactors with the capabilities reaching up to 20,000 L, there still remains a significant need for innovation in bioreactor design [59,60]. Recently, Lei et al. and his team developed a scalable, physiologically relevant microreactor for stem cell expansion and differentiation [61–63]. It has been shown this microbioreactor can be utilized to manufacture human pluripotent stem cells (iPSCs) derived vascular muscle cells [64], neural stem cells [65], and expansion of human T cells [66]. Compared to conventional stirred bioreactors, this microreactor was fabricated using alginate hydrogel microtubes (AlgTubes) that can reach higher viability, higher purity, and higher yield [64].

Next, for the cultivated meat to have the texture and structure that resembles traditional meat, cells can then be incorporated into a supportive framework for growth and differentiation, Fig. 1C. This can be achieved by placing cells onto a scaffold, a biodegradable matrix that emulates the ECM microenvironment. Various biomaterials have been

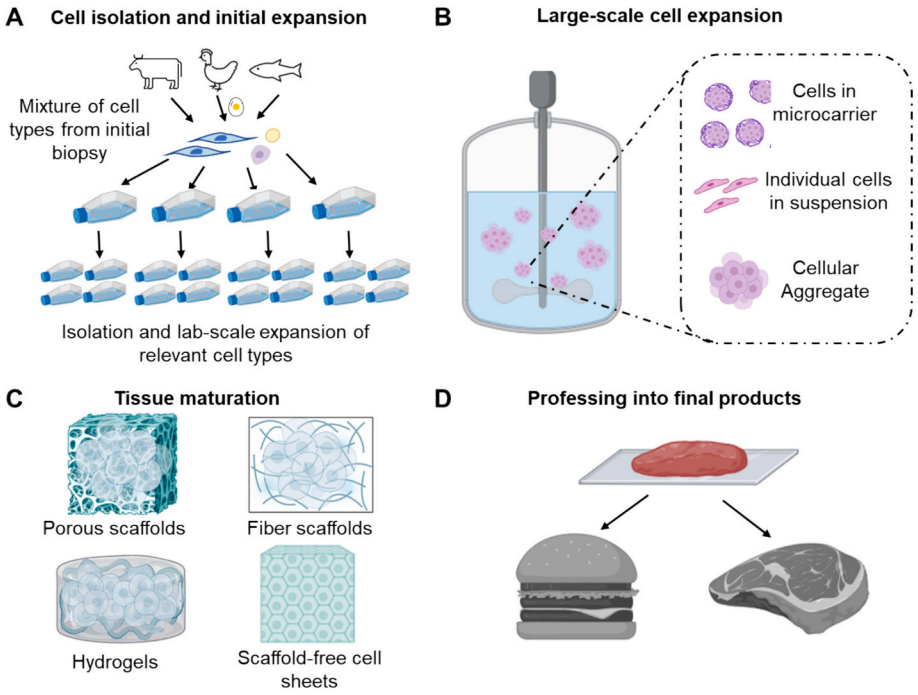


**Fig. 1.** Schematic illustration of the general process of cultivated meat production . A. Cell isolation and initial expansion for cultivated meat. In this step, cells were isolated from different sources, including chicken, cow, or fish. B. Large-scale cell expansion. In this step, cells were expanded through different methods. C. Tissue maturation. In this step, cells undergo maturation and differentiation on scaffolds. D. Processing into food products. The last step the matured tissue was converted into final product.

developed and reported, including 3D-printed biomaterials, edible polymers, and plant-derived materials [11,26,32,35,40,67]. Cells can thus undergo differentiation, maturing into muscle cells, fat cells, and connective tissues. The result of this step is a meat tissue with the desired structure and texture.

In the final product processing phase, the aim is to transform the cultivated cells into a product that is indistinguishable in taste and appearance from traditional meat [20,68], Fig. 1D. The matured meat tissue, once harvested from the scaffold, is subjected to familiar meat processing techniques, such as cutting and seasoning. It can also be further refined to produce specific items like burgers, sausages, or steaks. To ensure purity, any leftover medium or undesirable substances are removed, resulting in a final product ready for cooking and consumption, just like conventional meat.

In practice, the production process for cultivated meat can vary among companies and products, but a general sequence can be illustrated as in Fig. 1. Initially, relevant cells from the desired species are isolated, characterized, and banked for future applications. This often involves the creation of a stable, immortalized cell line. The second phase focuses on cell expansion to increase biomass, aiming for numerous cell doublings while maintaining the cells in a proliferative, undifferentiated state. In the third phase, tissue maturation, cells undergo differentiation and maturation, commonly on scaffolds. The media and bioreactor selection are pivotal in both the second and third phases, with distinct requirements for each. Certain products may require a final processing stage to convert the cultivated tissues into the end product.

## 3. Scaffolding strategies for cultivated meat

Scaffolds are essential for tissue engineering and cultivated meat production as they provide critical frameworks for cell proliferation and differentiation. There are several strategies that can be utilized for the fabrication of scaffolds, including microcarriers, porous scaffold, nanofibers, hydrogel, and cell sheet technology, Table 1. Microcarriers are small, often spherical beads that offer a surface for cell attachment, making them suitable for scaling up cell cultures in bioreactors. Microcarriers are typically used for large scale cell proliferation. Porous scaffolds, characterized by a network of interlinked spaces, are mainly utilized in the phase of tissue maturation. These types of scaffolds replicate natural ECM, enabling cell penetration and efficient nutrient and waste exchange. Porous scaffolds are typically constructed from synthetic polymers or a combination of natural and synthetic polymers. Hydrogels, in combination with the 3D bioprinting techniques, can create a 3D environment that supports cell proliferation and tissue morphogenesis. Conversely, the scaffold-free approach leverages the cells' intrinsic ability to self-assemble and create structured tissues without external support. This approach can produce tissues with native-like properties. Fig. 2 demonstrated the different types of scaffolding strategies for cultvated meat. The choice of the right scaffolding strategy depends on the intended application and desired tissue characteristics.

**Table 1**
Scaffolding strategies for cultivated meat advantages and disadvantages.

| Scaffold method | Advantages | Disadvantages | Types |
|---|---|---|---|
| Decellularized scaffolds | - Emulates native cellular habitat.<br>- Biocompatibility and bioactivity.<br>- Reduced environmental footprint. | - Limited scalability for large-scale production.<br>- Risk of immune responses with cross-species decellularization. | [30,32,66] |
| Microcarriers | - High surface-to-volume ratio for scaling.<br>- Precise control over growth factor delivery.<br>- Supports cell adhesion and proliferation. | - Variability in encapsulation efficiency.<br>- Limited to specific cell types.<br>- Some microcarriers may require additional coatings. | [68–76] |
| Porous scaffolds | - High porosity for efficient nutrient transport.<br>- Mimics ECM.<br>- Rapid cell penetration. | - Challenges in maintaining mechanical integrity.<br>- Difficulty in achieving precise control over scaffold degradation. | [24,28,78–81] |
| Nanofibrous scaffolds | - Exceptional surface-to-volume ratio for cell attachment.<br>- Optimal texture and structure.<br>- Mimics natural ECM. | - Complexity in scaffold fabrication.<br>- Precise control over fiber alignment can be challenging. | [30,31,82–99] |
| Hydrogels | - Mimics texture and consistency of real meat.<br>- Suitable for cell growth.<br>- Food-safe and biocompatible. | - Difficulty in achieving precise texture matching.<br>- Variation in mechanical properties. | [23,72,74,100–104] |
| Scaffold-free approaches | - Mimics natural cell self-organization.<br>- Avoids scaffold degradation.<br>- Reduces animal-derived materials.<br>- Sustainable. | - Challenges in controlling tissue structure.<br>- May require specialized equipment and techniques. | [105–109] |

### 3.1. Decellularized scaffolds

Recently, decellularized scaffolds have emerged as a preferred choice for fostering the proliferation of myogenic cells, primarily due to their remarkable compatibility with the intrinsic physiological characteristics of the cellular microenvironment conducive to growth [36]. Thus, decellularized scaffolds offer a compelling edge over their non-animal-derived counterparts in cultivated meat production and tissue engineering. This advantage stems from their capacity to closely emulate the native cellular habitat, providing an optimal substrate for the cultivation and maturation of engineered tissues and the production of cultivated meat products. The utilization of decellularized scaffolds not only harnesses the biocompatibility and bioactivity intrinsic to these materials but also aligns with the growing emphasis on sustainability in tissue engineering and cultivated meat production, as they can be derived from renewable sources and contribute to reducing the environmental footprint of these technologies [69]. Recently, Jones et al. demonstrated that by decellularizing spinach leaves, they could produce an edible scaffold with a vascular network. This network could potentially maintain the viability of primary BSCs as they develop into meat [34]. In another study, Tyden et al. demonstrated a rapid, food safe, decellularization procedure of broccoli florets to yield cell-free ECM scaffolds and evaluate them as cell carriers for cultivated meat [36]. Allan et al. recently reported the usage of decellularized amenity grass as a natural scaffold to support C2C12 myoblasts attachment, proliferation, alignment and differentiation [70]. Thus, decellularized scaffolds hold potential as a central element in cost-effective large-scale cultivated meat production. They are economical, natural, and edible, in addition, they not only act as a cell support but also enhance the nutritional qualities of the end meat product when utilized in suspension cultures. Despite the clear potential decellularized scaffolds hold, limitations still exist. Decellularized scaffold scalability is limited due to current bioreactor designs.

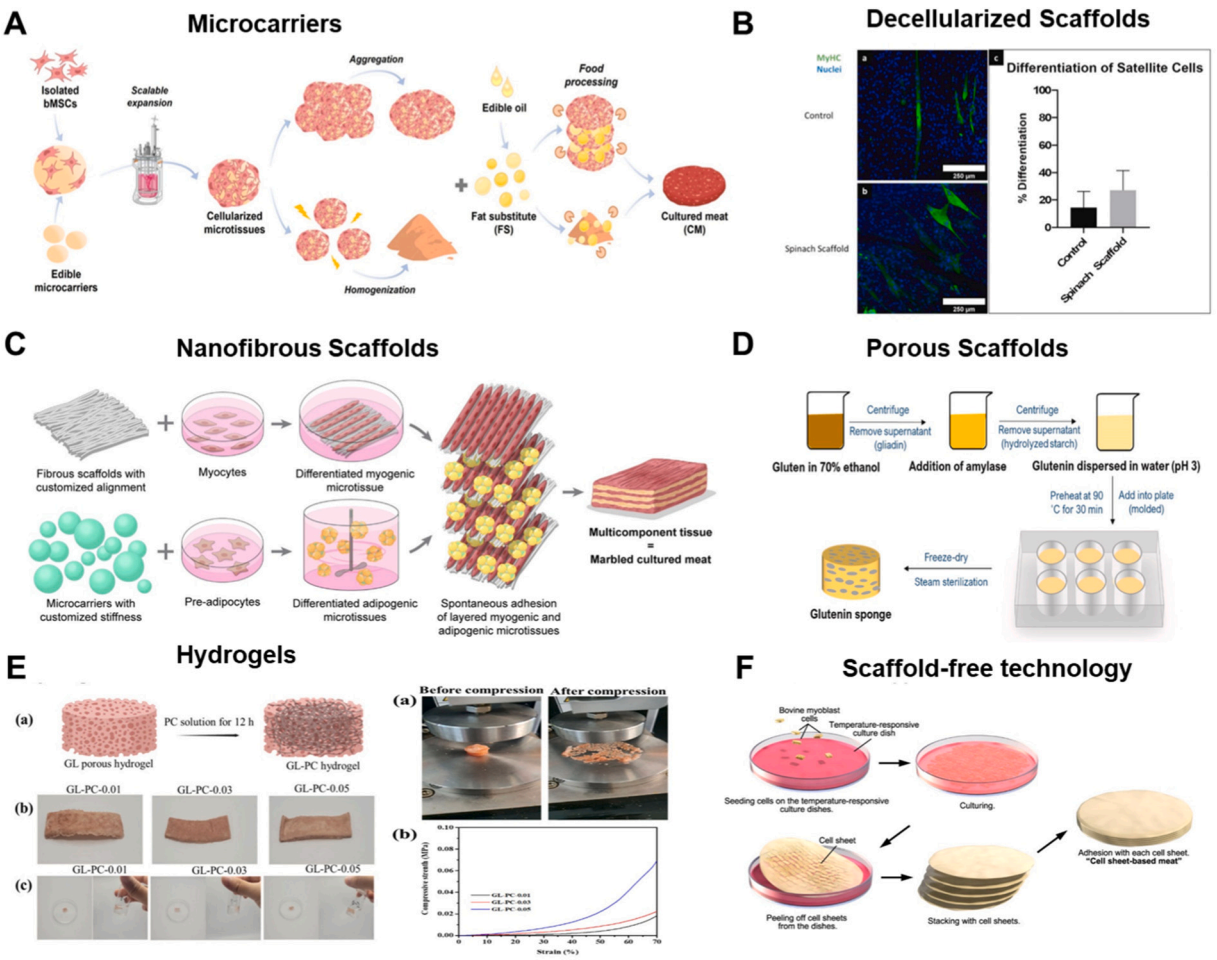


**Fig. 2.** A. Different stages in cultivated meat production [73], edible microcarrier-derived microtissues are first produced in a scalable bioreactor and then undergo processing approaches such as aggregation or homogenization. This cellular mass is then blended with oleogel fat substitutes and shaped by food processing techniques to create cultured meat (CM) prototypes. B. 14-Day differentiation of bovine satellite cells on decellularized spinach scaffold [34], C. Modular approach to marbled cultivated meat production using nanofibrous scaffolds [103], Myocytes and pre-adipocytes are cultured on electrospun fibers and emulsion-templated microcarriers, respectively. After harvesting post-differentiation, myogenic and adipogenic microtissues are stacked and spontaneously adhere to form intact multicomponent tissue or marbled cultivated meat. D. Diagram illustrating the extraction of glutenin from gluten and the creation of porous glutenin sponges [28]. E. Creation and characterization of GL-PC porous hydrogels for cultivated meat production [108], (a) GL-PC hydrogel synthesis—crosslinking, freezing, lyophilizing, (b-c) freeze-dried GL-PC structure and stability in PBS over time. F. scaffold-free cell sheet-based meat manufacturing [112].

Massive bioreactor systems would be imperative to produce the volume of satellite cells required [30]. The ideal suspension-style bioreactors also necessitate cost-effective designs in order to ensure that the cost of production is as low as possible [32].

### 3.2. Microcarriers

Microcarriers (MCs) are small, spherical polymeric beads, typically measuring between 100 and 500 μm, that are favored for their efficacy in cell encapsulation and in reducing cell necrosis [38]. The surface texture and porosity of these beads play a pivotal role in the encapsulation efficiency across different cell types, emphasizing their importance in MCs choice for cell culture [71]. Notably, their considerable surface-to-volume ratio makes them exceptionally suitable for scaling up muscle cell cultures, offering a distinct edge in muscle cell cultivation [72]. Recently, Yen et al. developed a cultivated meat platform that incorporates edible MCs and an oleogel-based fat substitute [73]. They expanded bovine MSCs on edible chitosan-collagen MCs, resulting in the creation of cellularized microtissues. Concurrently, an oleogel system using plant protein was fashioned to imitate the look and texture of beef fat. Merging these cellularized microtissues with the synthesized fat substitute allowed this team to present two cultivated meat prototypes: a layered version and one resembling a burger. The former displayed increased rigidity, while the latter mimicked the visual appeal and softer consistency of marbled meat. In another study, Norris et al. developed edible MCs using a unique method combining water-in-oil emulsions and an embossing technique, resulting in grooved surfaces [74]. These were designed to promote myogenic cell growth and differentiation within a bioreactor. Both the smooth and grooved MCs effectively supported cell proliferation and differentiation, leading to the formation of "microtissues." Notably, these aggregates could be transformed into a meat patty that retained its shape and browned when cooked. Moreover, Song et al. reported a large scale of porcine adipose-derived stem cells (ADSC) using MCs. The study optimizes ADSC culture conditions on microcarriers, ensuring high-density cell cultures [75]. In addition, Zernov et al. developed edible hydrogel MCs from chitosan and collagen [76]. These MCs were demonstrated to promote attachment and rapid proliferation for various cell types, including mouse skeletal C2C12 myoblasts, rabbit smooth muscle cells, sheep fibroblasts, and bovine umbilical cord MSCs. They could achieve full surface coverage within only a few days in culture.

Furthermore, MCs can be employed in cultivated meat production through supporting cell proliferation or incorporation into the final product. Recently, commercial companies began to develop edible MCs

intended for use in cultivated meat, for instance, Matrix Meats [77] and Omeat [78]. Although MCs present a simple solution for large-scale mammalian cell expansion without occupying much space, they may pose challenges in terms of cell dissociation and separation costs, the expense of the MCs, achievable cell densities, and potential effects on the nutritional and taste qualities of the end product. The margin cost of MCs for cell expansion depends on both the cost and the growth area per mass of MCs. The estimated marginal cost of MCs is prohibitively expensive at $3200 - $8400/kg of cells produced at current retail prices [79]. At this point, utilization of existing food biopolymers as materials for MCs fabrication, such as gum [80] and zein [81], would potentially mitigate the cost and ensure edibility.

### 3.3. Porous scaffolds

3D porous scaffolds, made from polymers and characterized by high porosity and an interconnected pore network, are of significant importance in the fields of tissue engineering and cultivated meat production [27,28,31,82]. These scaffolds act as important platforms for cell growth, tissue regeneration, and the delivery of bioactive compounds. Their high porosity ensures efficient transport of nutrients and oxygen, providing cells with an optimal environment to adhere, spread, and differentiate. Moreover, the tissue structure within these scaffolds closely resembles the natural ECM, fostering the integration and functionality of membranes. The sponge-like or foamy design of these scaffolds further enhances their efficacy. Such systems can be tailored to regulate tissue dimensions, mechanical attributes, and breakdown rates, rendering them appropriate for specific requirements. It has been reported by several groups that porous scaffolds can be fabricated using wheat glutenin [28], soy protein amyloid fibril [83], prolamin [32] and other edible porous proteins [84]. Traditional porous scaffold fabrication approaches, including freeze-drying, gas foaming, and melt molding, often use synthetic polymers, which should be replaced with edible biopolymers for the use of cultivated meat production. For example, Xiang et al. developed a 3D porous wheat glutenin scaffold using a unique water annealing method [28]. It was confirmed that the scaffolds, with pore sizes between 50 and 250 μm and compressive moduli from 0.5 to 1.9 kPa, remained stable for six months refrigerated, needing no toxic agents or animal-derived ECM coatings. They effectively supported muscle cell (C2C12 and bovine satellite) growth without extra adhesive proteins. Recently, Chen et al. developed 3D scaffolds using sodium alginate and gelatin, further enhanced with tea polyphenols (TP) to improve better biocompatibility and mechanical properties [85]. These scaffolds exhibited a porous laminar structure and maintained over 40 % porosity with minimal degradation. Evaluations with C2C12 cells showed promising adhesion and extension, suggesting the scaffold's suitability for cell growth. Rabbit skeletal muscle myoblasts (RbSkMC) cultured on these scaffolds not only adhered and extended well but also formed myotubes. Notably, the cultivated meat derived from these scaffolds closely matched real meat in appearance and texture [85]. Moreover, Chen et al. constructed a 3D edible scaffold by combining gellan gum (GG) and gelatin (Gel), which was then crosslinked with $Ca^{2+}$. Scaffolds enriched with higher $Ca^{2+}$ concentrations exhibited improved biocompatibility and cell adhesion. When cultured, these scaffolds closely resembled the texture and color of genuine meat products. Such findings underscore the biocompatibility and stability of the ionically crosslinked GG-Gel scaffolds for structured cultivated meat applications [86]. Although porous scaffolds have been investigated extensively in tissue engineering, the application in cultivated meat is limited by cost and scale considerations. Furthermore, porosity, pore size, and material composition are critical factors affecting cell survival and tissue development. The introduction of new biomaterials, such as edible biopolymers, could potentially mitigate costs.

### 3.4. Nanofibrous scaffolds

In the context of cultivated meat production, nanofibrous scaffolds are gaining promise due to their exceptional capabilities, including the ability to mimic nature ECM for muscle and fat tissue growth [87]. Their high surface-to-volume ratio enhances cell attachment, proliferation, differentiation, and maturation, which are important for producing meat-like textures and structures [88]. A variety of approaches, including electrospinning, melt blowing, and templating, are employed to fabricate these nanofibrous scaffolds, ensuring they meet the unique demands of cultivated meat. Electrospinning is one of the most popular techniques for the fabrication of nanofibers using a variety of materials, including Polycaprolactone (PCL) [89,90], poly(lacticco-glycolic acid) (PLGA) [91], polylactic acid (PLA) [92], gelatin methacryloyl (GelMA) [93,94], fibronectin [95,96], albumin [97,98], and gelatin [99–101]. Combination of biomaterials are also common for the fabrication of nanofibrous scaffolds, such as PCL/alginate [102] and PCL/gelatin [100].

Although nanofibrous scaffolds are predominantly used in tissue engineering, a number of studies have reported their application for myogenic and adipogenic cells [103], and muscle cells [104]. Kawecki et al. introduced an innovative approach to create marbled cultivated meat through engineering multicomponent tissue [103]. They utilized customized nanofiber and microbead scaffolds to enhance the growth of muscle cells and fat cells. This modular approach allowed myocytes to attach and proliferate on nanofiber scaffolds resembling skeletal muscle, while adipocytes attached on microbead scaffolds mimicking adipose tissue. These components naturally bind to create marbled cultivated meat, eliminating the necessity for extra crosslinkers. This technique holds potential for the scalable production of marbled meats across various species using different scaffold materials. In addition, Santos et al. explored the efficacy of cellulose acetate (CA) nanofibers, with and without annatto extract (CA@A), as scaffolds for cultivated meat and muscle tissue engineering [104]. The CA@A nanofibers demonstrated improved cell adhesion and boosted cell proliferation, promoting sustained cell growth. Morphological and mechanical analyses of these nanofibers revealed porous structures without pronounced fiber alignment. This research suggests that cellulose acetate fibers infused with annatto extract could present a cost-effective solution for muscle cell cultivation, making them potential scaffolding candidates for cultivated meat and muscle tissue engineering. In recent years, efforts have been made to enhance the large-scale production of electrospun nanofibers. Techniques such as multijet electrospinning, needle-less methods using diverse electrode designs, and high-capacity production of core-sheath fibers have been explored. Certain natural edible biomaterials show promise as nanofibrous scaffolds for cultivated meat applications due to their intrinsic biocompatibility, sustainability, cost-effectiveness, and ability to mimic the texture and nutritional qualities of traditional meats [34,35]. This makes them ideal biomaterials for scalable and eco-friendly meat production alternatives. Limitations for nanofibrous scaffolds include the complexity of fabricating this type of scaffold for food production and the difficulty of aligning fibers. Commonly used nanofibrous scaffold methods, including electrospinning, may prove difficult to scale up for cultivated meat production [98,99].

### 3.5. Hydrogels

Hydrogels are a hydrophilic polymer matrix with a large water absorbance capacity, where the matrix is cross-linked through either physical or chemical interactions. Over the past two decades, hydrogels have been one of the most common tissue engineering scaffolds due to their capacity to maintain a unique 3D structure [105]. Moreover, hydrogels can provide mechanical support for cell attachment and proliferation and simulate the native ECM. Their high water content, tunable mechanical properties, and biocompatibility make them conducive environments for cell growth and differentiation. In addition,

the high water content in these gels can mimic the texture and consistency of real meat while offering a stable structure for cell growth [105]. Thus, hydrogels are a rational biomaterial choice for cell cultivated meat. Hydrogels can be synthesized from various natural or synthetic polymers, allowing for customization based on desired attributes [27,79]. By offering essential nutrients and a support structure, hydrogels facilitate the organized growth of muscle cells [76], fat cells [106,107], and connective tissues [108]. For the application of cultivated meat, researchers have been exploring different types of hydrogels, such as alginate, collagen, and gelatin, to optimize the growth and texture of cultivated meat. Natural and food-grade hydrogel are commonly used for cultivated meat due to their biocompatibility, and ability to mimic nature ECM. For example, Rao et al. developed edible gelatin (GL)-based hydrogels using grape seed extract (proanthocyanidins, PC) for cultivated meat application [108]. The GL-PC hydrogels supported Bovine Satellite Muscle Cells (BSCs) growth, exhibiting suitable compressive properties and pore sizes (100–300 μm) for meat-like tissue formation. These hydrogels efficiently mimic muscle tissue, offering a suitable environment for cell development. In another study, Chen et al. developed programmable scaffolds that were fabricated from food-grade collagen hydrogel using an ice-templated freeze-drying approach [82]. They confirmed that this scaffold could not only provide sites for MSCs adhesion and proliferation, but also promote the oriented growth and differentiation of cells. In addition, Chen et al. fabricated an edible collagen hydrogel with linearly aligned microgrooves to direct MSCs alignment [109]. Although hydrogels have great potential for cultivated meat production, hydrogels also face challenges including achieving suitable mechanical properties to support cell growth and ensuring biocompatibility. Uniform nutrient distribution is crucial, but some hydrogels may inhibit efficient transport. The scalability of hydrogel production, cost-effectiveness, and long-term stability are also concerns. Ensuring that hydrogels are food-grade and free from animal-derived components is crucial for consumer acceptance.

### 3.6. Scaffold free approaches

Although scaffolds provide advantages for fabricating 3D tissues, such as enhancing nutrient transport and oxygen diffusion, scaffold-free methods can also address some of these challenges. Moreover, for cultivated meat production, scaffolds should be made from food-grade materials, which differ from those used in tissue engineering, and production costs should remain low. Gaining food-grade approval from regulatory authorities like the FDA is a significant challenge to the commercialization of cultivated meat. Consequently, recent research has explored cell sheet technology, which doesn't rely on 3D scaffolds and can be used to create scaffold-free 3D tissues [110,111]. Recently, Tanaka et al. demonstrated scaffold-free cell-based meat using cell sheet technology and characterized its texture and nutrients. They produced bovine myoblast cell sheets using temperature-responsive culture dishes (TRCDs) and 10 stacked cell sheets to fabricate three-dimensional tissue of 1.3–2.7 mm thickness [112]. The hardness of this cultivated meat increased during incubation and boiling, resembling natural meat. Despite this, the cell sheets contained about half the protein of beef. The method also allows for easy scaling to produce larger cell sheet-based meat, suggesting a potentially eco-friendly food product. In another study, Park et al. developed a cost-effective strategy for manufacturing cultivated meat by integrating edible gelatin microcarriers and myoblast cell sheets [113]. Furthermore, Choi et al. introduced a new food concept by cell powder meat (CPM), which has high nutritional content and a similar flavor to that of traditional meat. They revealed that this meat powder is produced 76 % more cost-effectively with less serum than the conventional culture medium and without a 3D scaffold [114]. This scaffold free CPM can also be prepared from various animal cells, including cattle, pigs, and chickens. Thus, the fabrication of CPM provides evidence for enhancing the scalability and reducing costs associated with cultivated meat production.

Cultivated meat practices have often used scaffolds to ensure that the product is a similar consistency to traditional meat. With a scaffold-free approach, it can be more challenging to control the resulting tissue structure. Additional limitations include producing a product with the same nutrients as natural meat. Using this method has led to products containing less protein than natural meat, but significantly more carbohydrates [107]. Suggestions have been made on how to remedy this, but further studies must be performed to confirm if an increase in protein and reduction of carbohydrates is plausible using a scaffold-free approach. Other studies have found that using scaffold-free approaches could lead to products that contain significantly more protein than traditional chicken and beef [109]. Consumers would need to take this into account when purchasing and consuming this type of product.

## 4. Types of biomaterials used as scaffold

Biomaterials for cell culture scaffolds can be sourced from animals, plants, or synthesized as polymers. While animal-derived biopolymers like collagen offer an environment similar to natural conditions, they pose ethical concerns in the context of cultivated meat due to their animal origin. Consequently, there's growing interest in exploring plant-based and microbial sources for biomaterials. Additionally, self-assembling peptides (SAPs) have the potential to be utilized for cultivated meat due to their versatility and ECM-mimicking properties. Fig. 3 summarized scaffold production materials from animal-derived biomaterials, synthetic polymers, plant-based materials, and self-assembling peptides (Table 2).

### 4.1. Animal-derived biomaterials

Animal-derived biomaterials, already part of natural meat and therefore edible, hold great potential as scaffolds for cultivated meat application. They offer a natural environment for cells to adhere, grow, and differentiate, thereby facilitating the development of tissues that mimic the texture and structure of traditional meat. Animal-derived ECM typically comes from the connective tissues of animals, predominantly composed of proteins such as collagen, elastin, and fibronectin, along with glycosaminoglycans. Derived from animal tissue, animal-derived ECM naturally possesses a composition and architecture that are suitable for mammalian cell growth. Furthermore, animal-derived ECM closely resembles the texture and structure of traditional meat, potentially offering a more authentic meat-like experience. Primarily, collagen and gelatin have gained prominence in this area. Their popularity extends beyond the food sector to the pharmaceutical and cosmetics industries, thanks to their biocompatibility, easy degradation, and minimal immune response in humans [115]. Given its matrix-like properties, animal-derived collagen emerges as a promising scaffold choice [116]. Collagen has been extensively used for tissue engineering and is an ideal material for cultivated meat. This is due to its robust and tunable mechanical properties, versatility across different applications depending on the specific collagen type and its modifications, and its ability to facilitate cell adhesion, growth, and differentiation [27].

Recently, Zheng et al. reported an approach to integrate smooth muscle cells (SMCs) into a collagen gel-based meat model [117]. They found that in this model, the inclusion of SMCs reduced pressure loss, increased collagen levels, and resulted in firmer, springier, and chewier meat compared to controls. These findings suggest that by producing ECM proteins, SMCs notably enhance cultivated meat texture. Furthermore, Zernov et al. developed edible hydrogel MCs from chitosan and collagen, the materials that are known for their versatility in tissue engineering [76]. The obtained composite MCs have a uniform spherical shape of 571 μm diameter, a smooth surface, and suitable mechanical properties. These MCs facilitate the attachment and rapid proliferation of mouse skeletal C2C12 myoblasts, rabbit smooth muscle cells, sheep fibroblasts, and bovine umbilical cord MSCs, achieving complete coverage of the carrier surface within only a few days in culture [76]. In

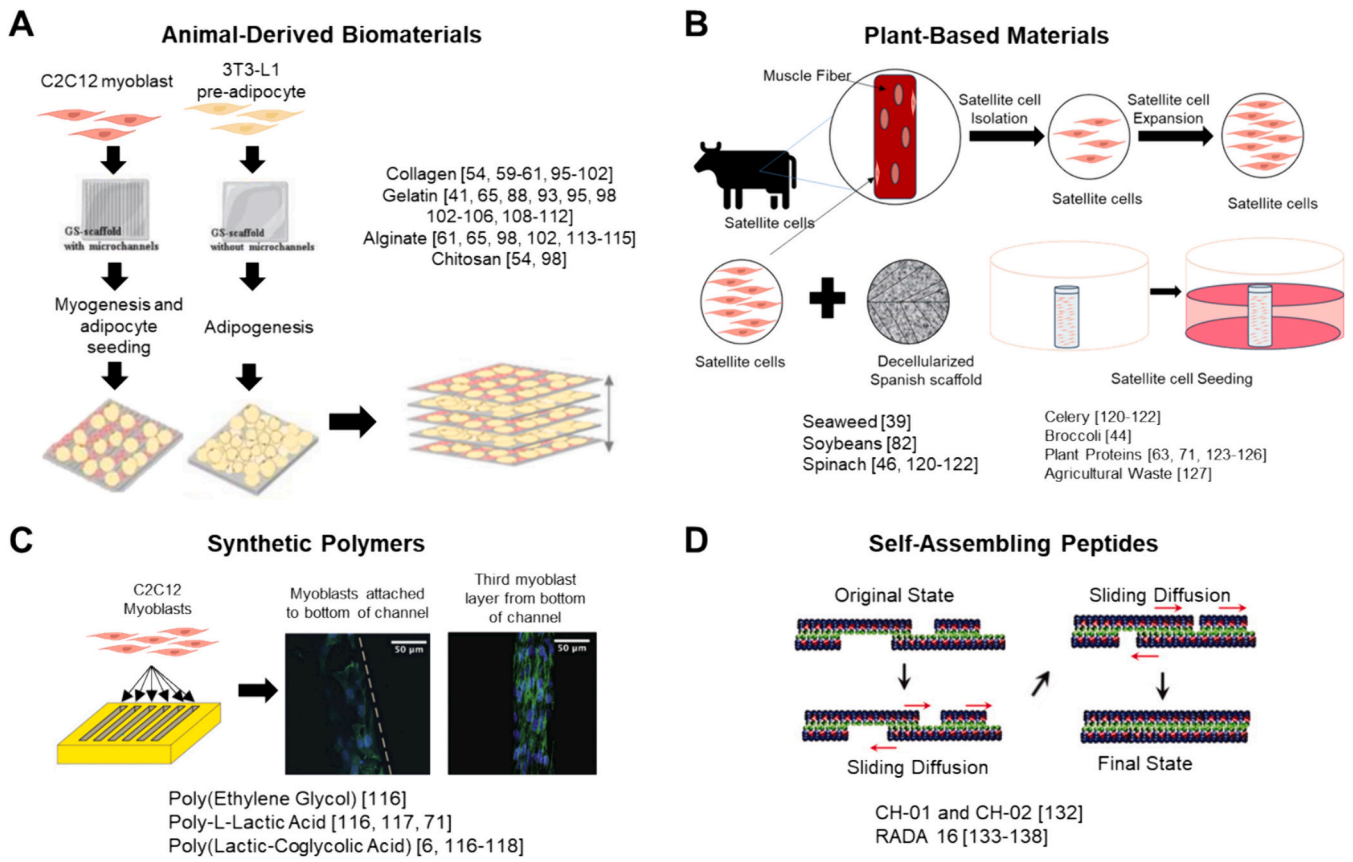


**Fig. 3.** Scaffold production materials from animal-derived biomaterials, synthetic polymers, plant-based materials, and self-assembling peptides.
A. Figure adapted from Li et al. to show the schematic of the gelatin-soymilk scaffold production and the resulting fat-containing cultivated meat [121]. B. Figure adapted from Jones et al. to show the process of isolating and seeding primary bovine satellite cells onto a scaffold made up of decellularized spinach [34]. C. Figure adapted from Hume et al. to show the ability of muscles to grow in layers when using a PEG-RGD hydrogel [132]. D. Figure adapted from Yokoi et al. to illustrate the ability of RADA 16 self-assembling peptides to alter their structure [152].

**Table 2**
Advantages and disadvantages for cultivated meat scaffold types.

| Scaffold type | Advantages | Disadvantages | Citations |
|---|---|---|---|
| Animal-derived biomaterials | - Edible<br>- Biodegradable<br>- Biocompatibility<br>- Mimics natural meat ECM<br>- Tunability | - Uses animal products<br>- Expensive<br>- Less reproducible | [22,23,27,33,72,77,80,108,110–129] |
| Synthetic polymers | - Easy to use<br>- Cheap to produce<br>- Different configurations are possible<br>- Easy replication | - Not edible<br>- Not biodegradable or slow degradation<br>- Additional cost and time of production<br>- Little cultivated meat research performed | [11,86,130–132] |
| Plant-based materials | - Edible<br>- Vascularized<br>- Low cost<br>- Biodegradable<br>- Environmentally friendly<br>- Mimic animal-based ECM | - Disconnect in mechanical properties<br>- Necessity to tune scaffolds | [30–32,78,86,133–141] |
| Self-assembling peptides | - Easy to manipulate<br>- Variable structures | - Little cultivated meat research done<br>- Expensive to produce | [142–154] |

another study, Li et al. engineered a 3D edible scaffold made of chitosan, sodium alginate, collagen, and gelatin (CS-SA-Col/Gel) [118]. The 3D 2-CS-SA-Col1-Gel scaffold, created using freeze-drying and electrostatic interactions, effectively supports porcine muscle cell growth, leading to a stable cultured cell meat (CCM) model with strong adhesion sites [118]. Moreover, this structured CCM model exhibited similar textural properties (like chewiness and resilience) and appearance to those of fresh pork. Collagen-based scaffolds encompass microcarriers [76], porous structures [82,118], hydrogels [118], and films. Furthermore, several bioprinting techniques have incorporated collagen [31,37,119–121]. Particularly, collagen I can interact with bFGF, acting as a gradual-release reservoir. Although all collagen types possess a characteristic triple-helix formation, they display variations in amino acid sequences and overall arrangements. In connective tissues, collagens are predominantly found as fibrils, though other configurations are also prevalent.

In addition, gelatin, which was derived from partially broken-down collagen, comprises beneficial polypeptides essential for various body

functions [122–124]. Gelatin has demonstrated efficacy as a scaffold for adipogenesis in cultivated meat. By layering gelatin with soymilk scaffolds, a blend of animal and plant-derived scaffolds can produce cultivated meat with varied cell types [125]. Recently, Park et al. developed a method for advanced cultivated meat using fish gelatin's MAGIC powder and myoblast sheets [113]. This powder, with its edible gelatin microsphere (GMS) structure, exhibited variations in morphology and bonding based on crosslinking. They found that GMSs significantly enhanced the myoblast sheet culture, yielding more effective meat-like cell sheets than traditional approaches. Given their diverse surface properties determined by crosslinking, GMSs were easily produced on a large scale. This team also concluded that the quality of cultivated meat, enhanced with GMS cell sheets, is comparable in tissue attributes to both soy meat and chicken breast. In another study, Lee et al. designed a coating matrix to enhance textured vegetable protein (TVP), aiming to emulate the core attributes of traditional meat [126]. They optimized the fish gelatin/agar matrix's microstructure by adjusting their ratio to encourage cell adhesion on TVP. This matrix, applied using a swift dipping process, resulted in a hybrid cultivated meat blending animal cells with plant protein. As these cells grew, their combined effects mimicked the texture, flavor, and taste of slaughtered meat, showcasing the potential for TVP to be a foundation in high-quality cultivated meat production [126]. Li et al. developed two hydrogel bioinks, ion-cured alginate-gelatin (AG) and light-cured GelMA-silk fibroin (GS), for 3D skeletal muscle tissue as potential cultivated meat [121]. By tweaking the bioink ratios, they identified the optimal blend for creating a 3D culture system using porcine skeletal muscle satellite cells (PMSCs). This addresses cultivated meat's limitation from PMSCs' in vitro adherent growth. Furthermore, Liu et al. introduced edible 3D gelatin microcarriers (PoGelat-MCs) for efficient cell growth and lab-grown meatballs production [127]. Using spinner flasks, PoGelat-MCs enabled scalable expansion of porcine and murine muscle cells, promoting spontaneous muscle formation without added myogenic agents. Utilizing a 3D-printed mold, they assembled pork micro-tissues into meatballs, closely mirroring traditional pork's texture and offering higher protein content.

Moreover, GelMA (Gelatin Methacryloyl) stands as a promising material in the cultivated meat sector due to its biocompatibility, tunability, and ability to mimic the ECM of natural tissues [26]. Derived from gelatin, GelMA possesses cell-binding motifs, facilitating cell attachment, proliferation, and differentiation – key factors for muscle tissue generation in cultivated meat -production [128]. When crosslinked, it forms stable hydrogels that can be tailored to match the mechanical properties of native tissues. With the potential for 3D bioprinting, GelMA can support the structured growth of muscle and fat cells, enabling the creation of meat analogues with textures resembling traditional cuts. For instance, Costantini et al. demonstrated that GelMA hydrogels effectively support the growth and differentiation of C2C12 myoblasts into myotubes [129]. Furthermore, hydrogels made from fish gelatin-based GelMA provided an environment for NIH3T3 embryonic fibroblasts, ensuring cell adhesion and proliferation [130]. Ebrahimi et al. showed patterned GelMA fibers could not only maintain C2C12 myoblast viability but also enhance myoblast alignment. Cells cultured on patterned GelMA fibers showed enhanced expression of myogenic markers, notably myosin heavy chain (MHC) and sarcomeric actin, indicative of myotube formation [131]. GelMA has also been used as a component in bioink with C2C12 myoblast in the printed structures that can survive over several weeks of differentiation. Although gelatin and GelMA possess similar benefits, GelMA's slower degradation might not be favorable in the context of cultivated meat. Therefore, animal-free gelatin might be a more suitable choice for cultivated meat scaffolding than GelMA.

While animal-derived biomaterials provide an environment that is similar to that of natural meat for cells to attach to and differentiate on, there remain concerns with this method. Using animal-derived biomaterials largely undermines the purpose of producing cultivated meat. Cultivated meat is intended to benefit the environment and reduce the cost of meat production. Animal-derived biomaterials still require the maintenance and sacrifice of livestock in order to provide the materials for scaffold production [23,108]. This ensures continued energy usage and money spent to upkeep the animals. Animal products commonly used for scaffold production, such as collagen, are also fairly expensive polymers, resulting in increased production costs [72]. Additionally, animal-derived biomaterials are less reproducible than other scaffolding methods due to the natural variability of animals [23].

### 4.2. Synthetic polymers

Synthetic polymers are made up of materials that are not readily found in nature. Synthetic polymers have been successfully used as scaffolds for other applications. Options for synthetic polymers include Poly(ethylene glycol) (PEG), poly-L-lactic acid (PLLA), and poly(lactic-coglycolic acid) (PLGA). PEG hydrogel scaffolds with multiple channels have been found to promote multiple layers of skeletal myoblast cell growth [132]. As skeletal muscle is a large portion of the consistency of meat, the ability of PEG to support this cell growth suggests that it may be a viable option as a scaffold for cultivated meat. PLLA and PLGA have been found to be sufficient scaffolds in terms of tissue engineering to restore large soft tissue defects [132,133]. The viability of PLLA and PLGA is largely due to the fact that these materials have a desirable vascularization which is important in cultivated meat scaffolding. These materials have been used for optimization of other scaffolds, as well [91]. PLGA can also be used in combination with other materials to produce porous scaffolds and other structures like MCs [11]. The benefit of PLGA is that it can assist in ensuring a scaffold has the ideal stiffness to mimic the material of interest [134]. The advantage of using synthetic materials as cultivated meat scaffolds is that different configurations can be easily produced at a low cost. Since the scaffolds are not natural, there is also the ability to replicate the scaffolds exactly, allowing for easy reproducibility. On the other hand, synthetic materials are not edible, and some have slow degradation or no degradation at all. Consequently, additional time and resources would be required to ensure that these materials are completely removed from the cultivated meat. Another concern is the limited research available on the use of synthetic materials as scaffolds for cultivated meat.

### 4.3. Plant-based materials

Plant-based materials, derived from plant-based sources like seaweed or soybeans, offer the advantage of not only edible and cost-effective but also facilitate cell adherence [35,135]. These materials provide structural support during the cell culture process and can be consumed along with the cultivated meat product. Researchers are exploring the use of decellularized plant tissues, such as spinach or celery, to create scaffolds that resemble the natural structure of meat [136–138]. These plant-based scaffolds offer an ideal environment for cell attachment, growth, and differentiation. One notable example is decellularized spinach leaves, which serve as vascularized scaffolds [34]. In addition, bovine cells were found to adhere to and differentiate on decellularized spinach leaf scaffolds while also ensuring a high viability after 14 days [34]. While these results were promising, the process would need further optimization for commercial-scale applications. Additionally, decellularized broccoli florets could be used as a scaffold in combination with MCs to provide additional encouragement for bovine cells to adhere and differentiate [36]. Alginate, a naturally occurring anionic polymer that can be obtained from brown seaweed, has been extensively investigated and used for tissue engineering and cultivated meat, due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations, such as $Ca^{2+}$ [139]. For example, Seo et al. developed cultivable alginate fibers for an ideal cultivated meat scaffold and production of hybrid cultivated meat [140]. By controlling the structure generated during the ionic

crosslinking process of alginate, cell adhesion was achieved at 82 %. In addition, Ianovici et al. assessed two plant-protein-enriched scaffolding compositions as 3D-printable platforms for BSCs maturation [31]. They then evaluated mixtures of pea protein isolate (PPI) and soy protein isolate (SPI) with RGD-modified alginate (Alginate(RGD)) as prefabricated mold-based and 3D-printed scaffolds for BSC cultivation. In another recent study, Chen et al. designed a 3D scaffold that was made of sodium alginate and gelatin with a surface coating of tea polyphenols (TP), ensuring high biocompatibility and robust mechanical support [85]. Tahir et al. synthesized and created methacrylate alginate (AlgMA) and methacrylated alginate and arginyl-glycyl-aspartic acid RGD conjugates (AlgMA-RGD) to enhance C2C12 cell adhesion for the application of cultivated meat [141]. Although alginate is a popular biomaterial for the application of tissue engineering, including cultivated meat, however, its application is limited due to concerns about limited cell adhesion and rapid degradation. For cultivated meat applications, these challenges can be critical as they might affect the efficiency, quality, and yield of the final product. However, these can be addressed by blending alginate with other polymers or using modified versions of alginate to improve its properties for meat cultivation.

Moreover, it has been reported that plant proteins, with their diverse structures and compositions, might contain active domains hypothesized to replace the animal-based ECM for cultivated meat applications [142]. Wei et al. developed 3D porous scaffolds by cross-linking soy protein amyloid fibrils. These scaffolds allowed C2C12 mouse skeletal myoblasts to proliferate and differentiate without the need for additional cell adhesive proteins or coatings [83]. Moreover, amyloid fibril were utilized to crosslink with fibril to form aerogels that are suitable for cell growth [143]. Several studies demonstrated the use of textured soy protein as a novel cultivated meat scaffold that can support BSCs and MSCs attachment and proliferation [91,144,145]. Agricultural waste has recently been looked at for cultivated meat scaffolding. Decellularized jackfruit rind and corn husk have been found to be slightly stiffer than natural meat while still providing a structural basis for BSCs and avian cells to adhere and grow on [146]. More research would need to be conducted to confirm the usage of agricultural waste as a viable scaffold, but it offers a low-cost scaffolding option. Overall, plant-based scaffolds are advantageous due to the fact that they are edible and degradable non-animal derived materials. They help mitigate the environmental impacts associated with traditional meat production and can mimic the nutrient pathways in cells due to their vascularization. However, challenges with plant-based scaffolds include discrepancies in mechanical properties and the need to fine-tune the scaffolds to ensure desired characteristics.

### 4.4. Self-assembling peptides

Self-assembling peptides (SAPs) have been investigated and utilized for tissue engineering scaffolds and 3D bioprinting materials due to their versatility and ECM-mimicking properties [147,148]. SAPs are made up of monomers that are able to conform into structures according to the environmental features around them allowing for use in a variety of functions [147,149]. Self-assembly can be tailored for specific applications by changing the nature of peptide sequences, while more robust and complex materials with advanced design features are feasible by simple crosslinking with biological macromolecules [150]. Amino acid side chains offer sites for chemical alterations, producing diverse supramolecular structures and adaptable hydrogels. These hydrogels can gain properties like shear-thinning, bioactivity, self-healing, and shape memory, expanding self-assembling peptide material applications. Supramolecular peptides can structurally assemble into nanofiber hydrogels based on distinctive building blocks. These hydrogels serve as nanomorphology-mimetic scaffolds for tissue engineering. Biochemically, peptide nanofiber hydrogels can have bioactive motifs and factors either covalently tethered or physically absorbed to them, providing various functions based on physiological and pharmacological needs [150]. Self-assembling peptides known as CH-01 and CH-02 have been used to produce hydrogels that can act as scaffolds. The hydrogel was found to successfully mimic ECM and display a nanofibrous structure similar to that of collagen in natural meat. The hydrogels were able to support the adherence and proliferation of muscle myoblasts [151], suggesting a viable option in cultivated meat scaffolding. RADA 16 is a synthetic amphiphilic peptide designed to self-assemble in a controlled way into fibrils and higher order structures [152,153]. Recently, Dzierżyńska et al. constructed a 3D system based on RADA 16 peptides that could improve fibroblast cell proliferation and enhance wound healing [154]. These hybrids are not cytotoxic, and stimulate skin cells to grow, which can potentially serve as scaffolds for cells. RADA 16 was also shown to promote the growth and osteogenic differentiation of rabbit dedifferentiated fat cells when exposed to osteogenic factors in the medium [155]. After 14 days of culture, these cells produced an ECM enriched with calcium. Gao et al. developed a RADA 16 scaffold through adding angiogenic polypeptide SVVYGLR to the carboxyl terminal of RADA 16 to enhance MSCs differentiation [156]. RADA 16 combined with methylcellulose has been utilized as a bioink for printing scaffolds infused with human or murine MSCs [157]. The 3D-printed structure with murine MSCs facilitated adipogenic differentiation and subsequent lipid buildup upon medium induction.

Although SAPs have been utilized for the application of tissue engineering, the use of SAPs in cultivated meat remains unexplored in existing literature. One potential reason for this may be related to the high cost of conventional peptide synthesis which could limit further research being performed. Potential strategies that could reduce the cost of SAPs production for cultivated meat scaffolding include optimization of current approaches by using recombinant organisms. Cell-free systems [158,159] which bypass the need for microbial hosts, present another potential method for SAP production.

## 5. Challenges and future perspectives

Despite recent progress, researchers continue to face challenges in harnessing the full potential of biomaterials for tissue engineering and cultivated meat. In this section, we will discuss the key obstacles in the development of scaffolding materials. Subsequently, we will summarize the current challenges in commercialization of cultivated meat, including but not limited to, scalability, edibility, cost, animal-free medium, consumer acceptance, regulatory, and food safety (Table 3).

### 5.1. Scaffold design consideration

First, identifying the ideal scaffolding biomaterial for cultivated meat is challenging; they must be biocompatible, ensuring that they support cell attachment, proliferation, and differentiation without causing adverse reactions or inducing toxicity. Additionally, these materials should imitate the structure and texture of traditional meat, necessitating appropriate mechanical properties. They should be adequately porous for nutrient and oxygen transport yet sturdy enough to offer cellular support. Existing materials often struggle with structural integrity, promoting cell growth, and ensuring differentiation. The degradation rate of these scaffolds should ideally align with tissue formation to avoid hindering development. In addition, scaffolds like those mentioned must have the mechanical strength to handle high shear stress caused by the culture medium running in the bioreactors. These need to be created with porous structures or soft elastic surrounding gels that can manage shear stress; this is not very easy to achieve. Enhancing cell adherence to biomaterial scaffolds is essential for tissue formation, as is cell functionalization. Developing novel techniques that are compliant with food safety requirements and functionalizing materials like alginate, PCL, and PLGA with ECM motifs are challenging. It is crucial to make sure that these changes do not compromise the final cultivated meat product's nutritional value or safety. Current biomaterials cannot perfectly mimic the taste and nutritional attributes of

**Table 3**
Scaffolding biomaterials challenges for cultivated meat.

| Challenges | Description | Ref |
|---|---|---|
| Biocompatibility | biomaterial must be compatible with the cells, not cause adverse reactions. It should support cell attachment, proliferation, and differentiation without inducing toxicity or immunogenic responses. | [11] |
| Structure and texture | Have the right mechanical properties, porous for nutrient transport and cell infiltration, provide necessary support. | [67] |
| Nutrient transport | Thick tissues require a way to transport nutrients and oxygen to cells deep within the scaffold, which is a challenge without a built-in vascular system. | [11] |
| Integration with cells | Ensuring that cells uniformly integrate and grow within the scaffold is crucial. Uneven cell distribution can lead to non-uniform tissue development. | [26] |
| Scalability | The biomaterials need to be produced at a large scale to meet the demands of mass meat production. | [199] |
| cost | For cultivated meat to be commercially viable, the cost of producing biomaterial scaffolds needs to be low. Some materials, especially those with unique properties, can be expensive to produce. | [211] |
| Animal free medium | Culture medium is crucial and represents >99 % of the expenses in cultivated meat production. However, research and development have been limited by the absence of serum-free media that supports robust cell expansion across multiple passages. | [186,211] |
| Regulatory aspects & food safety | Any new materials used for food production will need to undergo rigorous testing and approval by food safety authorities, which can be a lengthy and unpredictable process. | [29] |
| Consumer acceptance | While many are open to trying and regularly buying CM, only half would pay more. For commercial success, it's crucial to mimic traditional meat's taste, texture, and appearance. | [29] |

traditional meat. To make cultivated meat a viable alternative, these challenges must be addressed.

### 5.2. Scaffold edibility, scalability and cost

For commercial success, biomaterials must be cost-effective and scalable to meet mass production needs. It's also essential for these tissues to have an in-built system for nutrient transport, given the thickness of some tissues. To genuinely distinguish cultivated meat from its traditional meat, the biomaterials should be free from animal-derived components. Since different meats possess unique textures, the tunability of these biomaterials is essential to mimic the traditional meat's taste and sensory. In principle, the scaffold utilized for cell growth and differentiation needs to be edible since the final engineered muscle fat tissue will likely have intricate tissue-level structures that may be nearly impossible to separate from their original scaffold. However, among the current scaffolding biomaterials that support myogenic and adipogenic differentiation, such as collagen, gelatin, hyaluronic acid (HA) [160,161], fibrin, alginate, chitosan, PEG [162], PLGA [134,163,164], PCL, and decellularized tissues, only alginate, gelatin, and PEG are edible and commonly used in food applications. Although decellularized plant tissues are one of the ideal scaffolds for cultivated meat due to their edibility, their usage may be limited to plant or fungal sources. Moreover, it may also face challenges in both scalability and cost-efficiency. While synthetic polymers, such as PEG, PCL, and PLGA are approved for medical use, including drug delivery, sutures, and dermal fillers, and can be produced at a low cost, it remains uncertain whether consuming large quantities of these polymers in cultivated meat products will be safe for food consumption. Although naturally derived biomaterials are likely safe for consumption, they are conventionally derived from animal sources, which limits their usage in cultivated meat production [165–167]. Furthermore, identifying methods for decellularizing plant tissues or edible polymers to produce scalable and sustainable scaffolds is challenging. These methods must be cost-effective, suitable for large-scale production, and environmentally friendly.

In addition, a variety of edible biopolymers, including polysaccharides, proteins, and lipids, have been used as ingredients in meat products. However, only alginate and gelatin have been widely used in tissue engineering scaffolds [168,169]. It is only very recent that bioengineering researchers have begun to adapt the edible biopolymers for cultivated meat applications. As there's a growing interest in sustainable and cost-effective scaffolds, researchers are now creating ECM-mimetic scaffolds with bioreactor-friendly designs (like porous hydrogels, fibers, and microcarriers) using different edible biopolymers. They are also evaluating biocompatibility for materials like carrageenan [170], pectin [171,172], cellulose [172,173], guar gum [174], gellan gum [175–177], xanthan gum [178–180], konjac [181–183], protein isolates from soy or corn (zein) [173], and starches [92,184,185]. A challenge with these biopolymers, especially polysaccharides, is their general lack of cell adhesiveness. This necessitates either chemical modification for cell adhesion or the creation of composites inclusive of a cell-adhesive protein. However, so far, only pectin, cellulose, gellan gum, and soy protein isolate have been explored for producing engineered muscle tissue. In addition, starches have been recently used for the application of cultivated meat [92,185].

### 5.3. Cell culture medium consideration

The cell culture medium is crucial for cultivating meat, yet it possesses significant challenges for cultivated meat production. One of the main reasons is that cell culture medium comprises the majority (>99 %) of the cost of current production systems [186,187]. Majority of current culture media used for cultivated meat is the same as that for lab cell culture, consisting of high-cost pharmacological grade ingredients. Transitioning to food grade ingredients could potentially reduce costs. In addition, meat cell cultivation, especially BSCs, traditionally uses fetal bovine serum (FBS), a costly, inconsistent, and unsustainable component that goes against cultivated meat objectives. Furthermore, formulations of amino acids and protein micronutrients (such as growth factors) suitable for cell-culture media are not yet produced at scales appropriate for food production and are perceived to be quite costly [187]. Particularly, animal cell-culture media typically contains a specific mix of sugars (like glucose), up to 20 essential and non-essential amino acids, fatty acids, phosphate, trace minerals, and a variety of vitamins, hormones, and cytokines, which are collectively referred to as growth factors. Many of these components are not yet produced at scales that are suitable for food production [187]. Thus, there is an emerging need for the development of an affordable medium, free of animal components, that is capable of maintaining proliferation and differentiation of BSCs. Recently, several research groups have developed serum-free medium for expansion of BSCs. For example, Stout et al. developed a low-cost serum free media through the addition of a single component, recombinant albumin to B8. The modified medium was demonstrated to be a suitable medium for long-term satellite cell expansion without sacrificing myogenicity [186]. The same group recently developed Beefy-9 serum-free medium for bovine satellite cell culture. Beefy-9 was altered by replacing recombinant albumin with rapeseed protein isolate (PRI), a bulk protein solution obtained from agricultural waste [188]. Moreover, Mitic et al. reported a reduced defined medium for adipogenic differentiation [189]. They found out that only insulin and rosiglitazone are necessary in both defined animal component-free (DMAD) and serum containing medium, with DMAD outperforming FBS. In another study, Yamanaka et al. developed a

serum-free medium that contains nutrients extracted from microalga and cell-secreted growth factors, which promoted the proliferation of bovine myoblasts, the main cell source for cultured beef [190]. Several other groups also developed different types of serum-free medium for the proliferation of muscle cells [191], bovine myoblasts [192], and BSCs [193].

Although there is a growing number of researchers that are working on the development of serum-free medium, the industrial level serum-free medium is not available and still under investigation and optimization. Much insight into strategies for achieving media formulations with these qualities can be obtained from knowledge of conventional culture media applications and from the metabolic pathways involved in myogenesis and protein synthesis. Successful production of cultivated meat requires media that is food grade with minimal cost, can regulate large-scale cell proliferation and differentiation, has acceptable sensory qualities, and is animal- ingredient free. Additionally, the principles used to optimize media for large-scale microbial fermentation processes that produce lower-value commodity chemicals and food ingredients can also be instructive.

### 5.4. Bioreactor considerations

A bioreactor is an important component for scaling cell production in the application of tissue engineering and cultivated meat. By offering a biological environment for cell growth and development, bioreactors allocate significant volume for cell expansion, nutrient diffusion and mechanical support. These advantages allow for larger-scale cell culture while simplifying medium recycling and replacement during the proliferation stage. The optimal culture conditions can be controlled by monitoring the oxygen, pH value, and medium. Typically, a fed-batch system is employed to provide nutrients during the culture process. Although different types of bioreactors, including stirred-tank bioreactors, perfusion bioreactors, and hollow fiber bioreactors (HFBs), have been widely developed and utilized at pharmaceutical companies, the required scale of cell expansion for cultivated meat production is orders of magnitude larger than that for tissue engineering applications [194,195]. Thus, the relatively low working cell density ($10^5$–$10^6$ cells/mL) and modest working volumes (50 L) of currently used bioreactors may face significant scalability limitations [196–198]. Compared to the approximated batch size of $5 \times 10^{10}$ cells produced by this method, 1 kg of muscle cells has around $3 \times 10^{11}$ cells. Among these different types of bioreactors, HFBs have exhibited the potential for expanding cells at a higher cell density ($10^8$–$10^9$ cells/mL) and higher volume [16,194,199,200]. HFBs have been utilized for expanding myoblast [199,201], bone-marrow derived MSCs [202–204], and adipose-derived stem cells [205,206]. However, high microfiber and cell densities could limit cell harvesting efficiency during intermediate expansion stages. This indicates that HFBs might be more practical during the cell differentiation phase of cultivated meat production, where the scaffold is intended to be edible and doesn't need separation from the differentiated cells. Recently, Lei et al. and his team introduced a scalable and physiologically pertinent microbioreactor designed for stem cell expansion and differentiation [61,65,66,207,208]. Studies have indicated that this microbioreactor is effective for producing iPSCs-derived vascular muscle cells, neural stem cells [65], and for expanding human T cells [66]. This AlgTubes bioreactor can achieve high purity, high viability and high yield (~$5.0 \times 10^8$ cells/mL in 10 days) [61]. Thus, once scaled up, this AlgTube bioreactor could potentially be a powerful alternative of traditional bioreactors in cell expansion for cultivated meat production (Fig. 4).

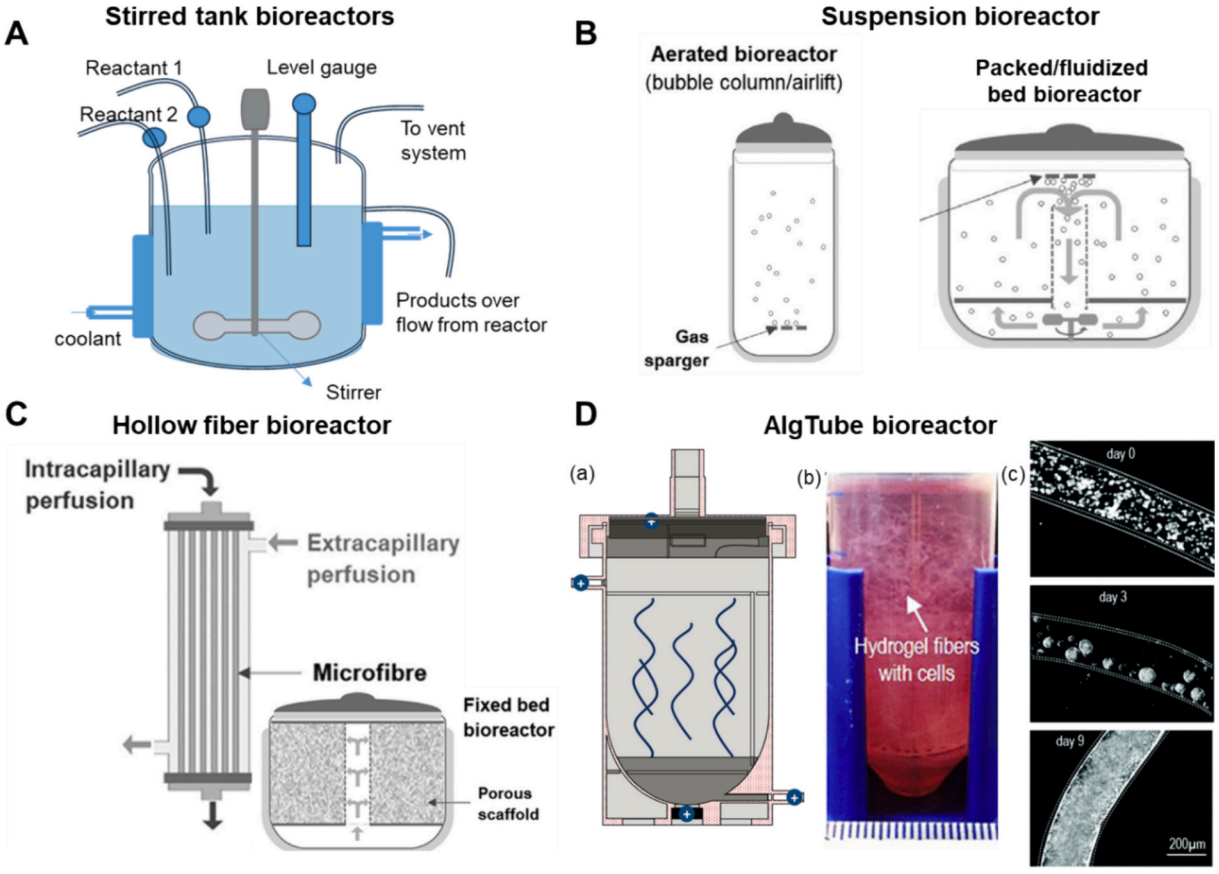


**Fig. 4.** A. Illustration of a stirred tank bioreactor. B. Illustration of a suspension bioreactor, aerated bioreactor and packed/fluidized bed bioreactor. [79] C. Hollow fiber bioreactor. Adapted from [79]. D. AlgTube Bioreactor. (a) Schematic illustration of AlgTube microbioreactor. (b) Collected alginate fibers with cells in suspension [208]. (c) hESCs in hydrogel tubes on day 0, 3 and 9. [63].

### 5.5. Regulatory and food safety

The launch of the first commercial cultivated meat product facilitated the transition to large-scale manufacturing facilities. This progression was evidenced by the approval and subsequent market introduction of the cultivated meat in Singapore in December 2020. After that, several countries, including the United States, UK, and Brazil, launched cultivated meat products. In June 2023, UPSIDE Foods and GOOD Meat are the first two companies in the United States to launch the first-ever "cell-cultivated meat" after clearing the final regulations [17,209]. Brazil is expected to begin producing and commercializing cultivated meat in 2024 [29]. In addition, Israel's Aleph Farms has submitted an application for regulatory approval to the Swiss Federal Food Safety and Veterinary Office (FSVO) with the goal of selling Aleph Cuts in Switzerland [210]. At this moment, the European Union, the United Kingdom, and Canada have an applicable regulatory framework related to cultivated meat. Typically, regulatory guidelines are expected to intersect between established norms for both the food and biomedical industries. Differences in regulatory frameworks across countries pose challenges for widespread adoption. As cultivated meat production advances, governments must strike a balance between fostering innovation and ensuring public health. Transparent regulatory standards are crucial for building consumer trust, driving investment, and ensuring the longevity of this promising alternative to traditional meat. Collaboration between scientists, policymakers, and industry stakeholders is essential for its successful integration into the food system.

The creation and commercialization of scaffolding biomaterials for cultivated meat faces regulatory and safety issues, consumer acceptance, religious concerns and ethical concerns and regulatory clearance. For instance, manufacturers may wish to incorporate genetically modified scaffolding materials or synthetic or engineered materials and peptides into cultivated meat production. Thus, any genetically modified or engineered materials may be subject to existing genetic modification regulations and labeling depending on jurisdiction. These are very crucial and depend on the safety and compliance of these materials [11]. Meeting additional regulatory requirements would result in longer review periods and higher compliance costs, and could influence consumer perception of cultivated meat products [28].

Moreover, scaffolds that are incorporated into the final product will be subject to food safety regulations, depending on the concentration and regional regulatory standards. Thus, scaffolds should be produced under Hazard Analysis Critical Control Point (HACCP) guidelines and maintain food standards to prevent food safety risks such as allergen cross-contamination. Thus, in order to reduce the potential risks, manufacturers should evaluate the safety implications of all scaffold materials and processing agents before using them.

## 6. Conclusion

In conclusion, significant progress has been made in scaffolding biomaterials for the application of cultivated meat. However, to achieve broad acceptance and successful commercialization, the challenges related to biomaterials, scalability, cost, texture, regulatory standards, and safety, need to be fully addressed. The integration of advanced biomaterials, technology, and improved bioprocessing has the potential to revolutionize food production. This review highlights the critical role of biomaterial developments in cultivated meat's success. The choice of biomaterials for scaffolding remains crucial, influencing the growth, texture, and overall quality of the meat produced. The role of scaffolds extends beyond mere structural support, also impacting nutrient flow and texture resemblance. As the field advances, interdisciplinary collaboration and innovation will be key in addressing challenges and realizing the transformative potential of cultivated meat in global food systems.

## CRediT authorship contribution statement

**Samantha Fasciano:** Investigation, Methodology, Project administration, Writing – original draft. **Anas Wheba:** Methodology, Writing – original draft. **Christopher Ddamulira:** Investigation, Writing – original draft. **Shue Wang:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Shue Wang reports financial support was provided by National Science Foundation (CMMI: 2143151). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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