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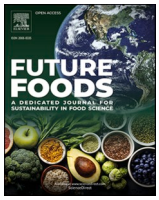

Bioreactor parameters and systems for cultured meat production

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ABSTRACT

Cultured meat (CM) is an emerging technology involving the use of animal cells to produce meat *in vitro*. It has the potential to replace or complement traditional meat production, offering a sustainable and ethical alternative to farmed animals. CM could provide sufficient meat to satisfy the growing demand for animal-based protein while addressing the negative impact of traditional livestock agriculture such as disease transmission, antibiotic misuse, poor animal welfare, and greenhouse gas emissions. But efficient industrial bioreactor systems, low-cost media, and high producing suitable cells are needed for CM technology to compete with or supplement conventional meat production. Bioreactors provide a controlled *in vitro* environment for the supply of nutrients and stimulus to the cells, which is necessary for cell expansion and differentiation on a large scale. In this review, we discuss the state of the art in bioreactor systems used for CM production, highlighting their characteristics and applications, advantages and disadvantages, the effect of key process parameters, and scale-up strategies. We also consider key performance indicators for the bioreactor-based large-scale production of CM.

1. Introduction

Cultured meat (CM) is an innovative sustainable technology involving the production of meat in a bioreactor. This is a promising approach to satisfy the demand for animal protein while addressing problems associated with traditional livestock farming (Bhat et al., 2017; Jara et al., 2023; Poore and Nemecek, 2018; Ritchie et al., 2023). Unlike conventional meat production, CM technology uses animal stem cells sourced without slaughtering, which are then expanded and differentiated in a controlled environment to produce meat-relevant tissues such as muscle and fat that can be processed into meat products ranging from minced meat to whole meat cuts.

Stem cells are the starting material for CM production, and these include muscle satellite cells (MuSCs) that can differentiate into muscle fibers, fibro-adipogenic progenitors (FAPs), which can differentiate into fat cells, and mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs), which can be differentiated into either muscle fibers or fat cells (Reiss et al., 2021). The cells are expanded in different bioreactor systems to produce a large number of cells that be differentiated by applying (bio)chemical or mechanical stimuli, forming either muscle fibers or fat cells depending on the cell type. The production of 10–100 kg of CM requires 10^{12}–10^{13} cells (Bodiou et al., 2020). Cell expansion in bioreactors sometimes involves adapted single cells or aggregates in suspension (Pasitka et al., 2023), but most systems are based on adherent cells cultivated on microcarriers, which provide scaffolds that mimic the extracellular matrix (ECM) *in vivo* (Andreassen et al., 2022; Norris et al., 2022; Yen et al., 2023). When the cells have been expanded to the required number, they can be differentiated to produce muscle fibers and fat cells that can be processed into minced meat. If whole meat cuts are required, the expanded cells are differentiated on 3D structured scaffolds, or 3D printing technology is used to create the structured product (Zheng et al., 2024). In most cases, two bioreactors are needed, one for cell expansion and the other for differentiation on 3D scaffolds (Chen et al.,

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2022). Combining cell expansion and differentiation in one bioreactor may reduce the cost of CM production (Schlößer and Nold, 2023).

Although cells and scaffolds are important for CM production, the key to industrial production relies on bioreactors. Bioreactors maintain the conditions that produce a well-defined and controlled environment for cell expansion and differentiation (Kulus et al., 2023). They must accommodate high-density cell cultures to produce meat at a scale and cost that is competitive with conventional meat. A bioreactor should balance factors such as oxygen transfer, shear forces, mixing, operational stability, reliability, and scale-up, guarantee sterility, and should be closely and efficiently integrated into the overall production system (Zhong, 2011). Bioreactors for CM are still predominantly adapted from the designs and technologies used in the brewery and pharmaceutical industries (Albrecht et al., 2024). These bioreactors can be operated in batch, fed-batch or continuous (perfusion) modes, which differ according to whether the cells are fed with fresh medium and/or whether waste or the final product is removed during the process (de Mello et al., 2023; Mitra and Murthy, 2022). Batch mode is the simplest. The bioreactor is filled with medium at the start of the process and the cells are allowed to reach maximum growth before harvesting, without the addition of more medium. In fed-batch mode, fresh medium is continuously or periodically added to the bioreactor, but there is no continuous removal of the product or waste. In continuous mode, fresh medium is added and spent medium is removed continuously, along with the final product and waste.

In this review, we describe the bioreactors used for CM production, focusing on critical process parameters, bioreactor design and applications, the advantages and disadvantages of each system, and strategies for bioreactor scale-up. We also provide guidelines for the development of optimal fit-for-purpose bioreactors for CM production.

2. Critical process parameters for CM production

2.1. pH

The pH of a solution is a measure of its proton (H^+) concentration, often described as acidity or alkalinity (Lim, 2006; Putnam, 2001). In biological systems, pH influences the ionization of amino acids, which determines the 3D structure of proteins. A change in pH may disrupt protein structure and cause a loss of function, which affects cellular processes such as respiration, nutrient uptake and metabolism, proliferation and differentiation (Ferreira et al., 2015; Putnam, 2001). For example, changes in pH have been shown to affect sensitive enzymes such as phosphofructokinase and the polymerization of actin and tubulin (Putnam, 2001). Furthermore, the availability of H^+ for chelation or release is an important mechanism of energy transfer and homeostasis (Ferreira et al., 2015; Putnam, 2001). To balance the availability of $H^+$with normal protein function, cells require mechanisms to buffer their internal pH, such as the generation of acid equivalents and the use of membrane-bound transporters for H^+, OH^- or HCO_3^- (Casey et al., 2010; Putnam, 2001). The extracellular pH of mammalian cells under physiological conditions is typically slightly alkaline (7.3–7.4) whereas the cytoplasmic pH can vary significantly between compartments, from 4.7 in lysosomes to 8 in mitochondria (Casey et al., 2010). Optimum pH values vary depending on the cell type and organism.

The pH is partially correlated with other critical process parameters. Carbon dioxide (CO_2) is continuously generated by cells and is introduced into *in vitro* culture systems. It easily diffuses through tissues and reacts with water to form carbonic acid, acidifying the solution but in deprotonated form (HCO_3^-) and acting as a buffer (Casey et al., 2010). Temperature affects the rate of auto-dissociation and can alter electrode sensitivity, which can cause errors in pH readings (AWE Ltd, 2020). Nutrients and metabolic products that contain charged functional groups (e.g., lactic acid) affect pH in a concentration-dependent manner.

2.2. Oxygen

Oxygen (O_2) is a necessary substrate for aerobic bioprocesses, and plays a key role in cellular respiration and metabolism. It acts as the final electron acceptor in the mitochondrial electron transport chain that generates adenosine triphosphate (ATP), the main energy currency of the cell (Pircher et al., 2021). The availability of oxygen in tissues, which is typically expressed as the partial oxygen pressure (pO_2), influences the growth, differentiation, and metabolism of cells (Carreau et al., 2011; Pircher et al., 2021; Place et al., 2017). The physiological range of available oxygen varies between species and tissues. In humans, the value is 8 mmHg (~1 % O_2) in the epidermis but 100 mmHg (~13 % O_2) in arterial blood (Carreau et al., 2011).

In muscle cells, physiological oxygen concentrations (2–10 %) have been shown to enhance myogenic differentiation by promoting the proliferation and fusion of myoblasts into mature myotubes (Pircher et al., 2021; Redshaw and Loughna, 2012). Low oxygen tension can activate hypoxia-inducible factors (HIFs) that regulate key genes for myogenesis (Itoigawa et al., 2010; Pircher et al., 2021). Similarly, the differentiation of adipocytes is influenced by oxygen. Low physiological oxygen levels (5 %) increase the expression of adipogenic markers such as peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα), and promote lipid accumulation and the maturation of adipocytes (Wang et al., 2018).

Although HIF signaling regulates the expression of multiple genes to prevent continuous hypoxia and restore physiological oxygen levels, hyperoxia can increase the production of reactive oxygen species (ROS) that dysregulate multiple signaling pathways such as nuclear factor E2-related factor 2 (Nrf2) or mitogen-activated protein kinase (MAPK), leading to cell injury and death (Alva et al., 2023).

2.3. Carbon dioxide

CO_2 is the primary product of oxidative phosphorylation during cellular respiration (Cummins et al., 2020). Under normal physiological conditions *in vivo*, CO_2 levels are regulated by highly sensitive chemoreception (e.g., a change of ~10 mmHg in pCO_2) that rapidly alters ventilation patterns (Cummins et al., 2020). CO_2 that diffuses across cell membranes reduces the intracellular pH, which affects enzyme activity and metabolism (Casey et al., 2010; Irfan, 2017; Pattison et al., 2000; Xing et al., 2017), and alters the levels of lactate and ammonium (Irfan, 2017; Pattison et al., 2000). Furthermore, high pCO_2 increases osmolality, which also influences ammonium and lactate production (Irfan, 2017).

Under *in vitro* conditions, CO_2 is often used to regulate pH because it dissolves in the culture medium to form carbonic acid, which dissociates into H^+ and HCO_3^- (Casey et al., 2010; Pattison et al., 2000). In tissue culture incubators, CO_2 is typically maintained at 5 % to mimic physiological conditions, which in mammalian cells is approximately equivalent to a pCO_2 of 50−70 mmHg at 37 °C (Pattison et al., 2000).

2.4. Temperature

Temperature is a critical process parameter in bioreactor systems and directly influences the biochemical and physiological processes of cultured cells. It affects enzyme activity, metabolism, growth rates, and the stability of biological products (Daniel et al., 2008, 2009; Scharnagl et al., 2005; Watanabe and Okada, 1967). Precise temperature control is necessary to maintain optimal cell functions, ensuring a consistent product quality (Pandey, 2022; Somero, 2020).

For most mammalian cells, the physiological temperature is ~37 °C, which is necessary to maintain homeostasis, optimal enzyme activity, and efficient protein folding (Campbell, 2008; Daniel et al., 2008; Guo et al., 2012). Deviations can lead to stress responses, altered metabolism, and lower viability (Guo et al., 2012; Reissis et al., 2013). A temperature of 37 °C is suitable for most mammalian cell cultures including the stem

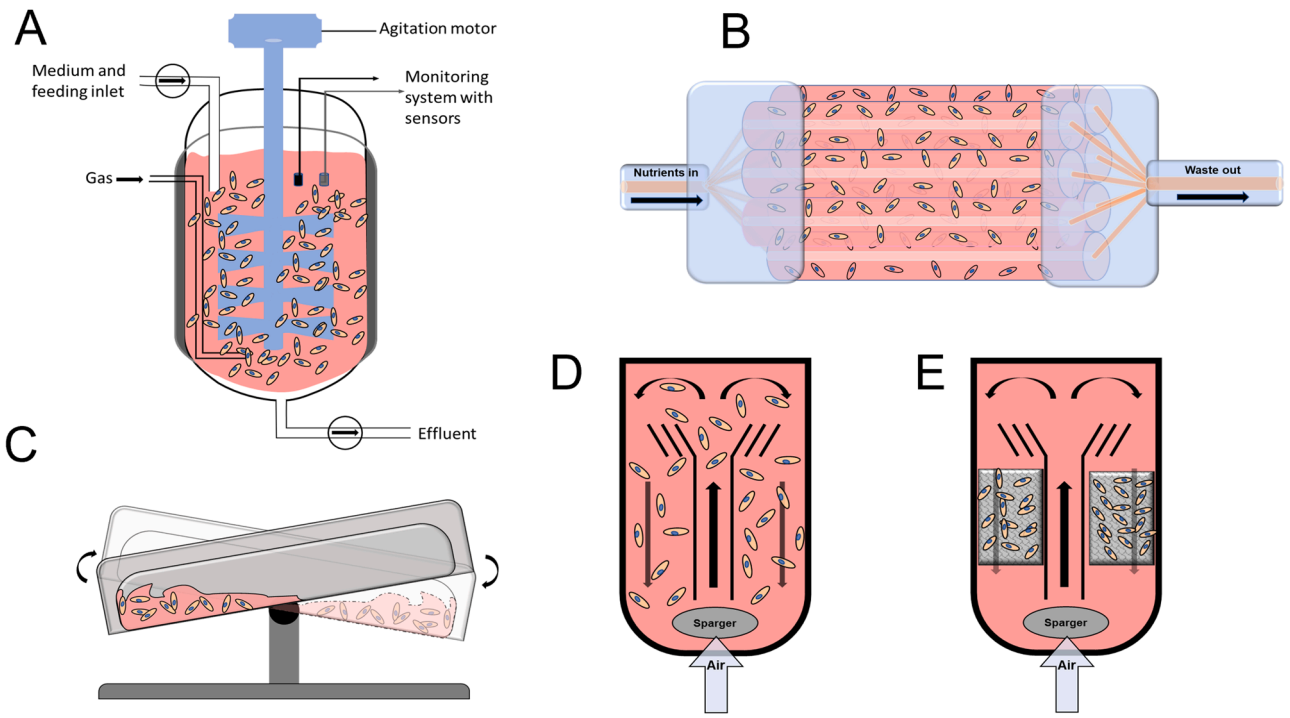

Fig. 1. Main bioreactor systems for CM production (cells not drawn to scale). (A) Stirred-tank bioreactor (STR). (B) Hollow-fiber bioreactor (HFB). (C) Rocking-motion bioreactors (RMB). (D) Airlift bioreactor (ALB). (E) Fixed-bed bioreactor (FBB).

cells used for CM production (Zidarič et al., 2020). Even so, lower temperatures in the range 30–35 °C can increase the yields of some proteins (O'Mara et al., 2018; Zidarič et al., 2020). In contrast to mammals, the body temperature of ectothermic animals has a broader range and is typically lower, with corresponding conditions required *in vitro*. For cultivated seafood production, the optimal temperature is 15–30 °C, depending on the species, and must be controlled precisely to ensure high quality of the final products (Courtenay Jr and Williams, 2004; Rubio et al., 2019; Xu et al., 2023).

Temperature deviations have significant effects on cell culture. Elevated temperatures (> 39 °C for mammalian cells) induce heat shock responses, leading to protein misfolding, reduced productivity, and cell death (Leber et al., 2012; Somero, 2020). Conversely, lower temperatures (< 34 °C for mammalian cells) can slow cell growth and metabolism, which may be desirable in some cases to improve product stability but can also reduce overall yields (Seebacher, 2009; Watanabe and Okada, 1967). Temperature variations can also influence other parameters. For example, O_2 solubility is temperature-dependent,

Table 1
Summary of pros and cons of bioreactors for CM production.

Bioreactor type	Advantages	Disadvantages	Max. working volume or area	Max. cell density	References
Stirred tank	• Bioreactor type highly characterized with high degree of knowhow • Simple design and highly adaptable • Enables easy scale-up • Can be used for cell expansion and differentiation	• Generates substantial shear stress • Detaching cells from microcarriers in bioreactor may pose problems for CM if microcarriers are not edible	250,000 L (under construction)	Not specified	(GoodMeat, 2022)
Hollow fiber	• Generates low shear stress suitable for CM production • Compact design that supports high cell densities in small volumes • Highly efficient diffusion process due to semipermeable membrane enabling proper nutrient delivery to cells	• Material of fiber may pose problems with cell harvesting for CM production • System results in formation of concentration gradients • May be difficult to monitor cell growth	2.1 m^2	>1 × 10^8 cells/mL	(Pan et al., 2023)
Rocking motion	• Allows efficient mixing with minimal shear stress • The use of disposable bag may simplify bioprocess workflow and reduce risk of contamination	• At very high volumes, efficiency of mixing and gas exchange may decrease • Scale-up could be challenging due to large installation space • Production cost may increase due to requirement for single-use bags	1000 L	1 × 10^7 cells/mL	(Kim et al., 2024)
Airlift	• Low shear stress due to air-driven agitation • Efficient oxygen transfer and mixing at small scale • Operational cost may be lower than for STRs	• Mixing problems may arise when cell viscosity is high • Scale-up is challenging • Potential foam formation	300 m^3	>2 × 10^8 cells/mL	(Li et al., 2020)
Fixed-bed	• Low shear stress and easy medium exchange • Allows direct CM production on scaffold	• May pose problems during cell harvesting if scaffold is not edible • Scaling up challenging	600 m^2	> 5 × 10^8 cells/mL	(Goral et al., 2024)

meaning that changes in temperature can influence oxygen consumption (Field et al., 1944; Fry and Hart, 1948).

2.5. Cell density

Under ideal conditions, the quantity of cells per unit volume would correspond to overall productivity. However, in a fixed space within a bioreactor, the number of cells that can be cultivated simultaneously is limited. Furthermore, the overall productivity of cells within a bioreactor generally begins to slow with increasing cell density above a certain threshold (Lavado-García et al., 2022). Assuming an optimized process, the cell densities that can be achieved in a bioreactor depend on the bioreactor type and cell type. In batch mode, high cell density (HCD) refers to cultures ranging from 10^6 cells/mL (Durocher et al., 2002; Lavado-García et al., 2022) to 2×10^8 cells/mL (Clincke, Mölleryd, Samani, et al., 2013, 2013; Lavado-García et al., 2022). To reach high densities and maintain optimal productivity, cells require sufficient access to nutrients, efficient gas exchange and removal of important waste products like ammonia and lactate, while maintaining other parameters at optimum levels. Cell density and productivity are therefore dependent on multiple parameters. However, per-cell productivity is compromised at high cell densities even when efficient fed-batch or continuous bioreactor systems are used, suggesting that cell-specific mechanisms may limit the productivity of the system (Fuenmayor et al., 2019; Henry et al., 2004; Lavado-García et al., 2020, 2022).

The cell density effect in bioreactors remains an important challenge. However, the removal of extracellular vesicles and the overexpression of UDP-glucose ceramide glucosyltransferase significantly increased the production of virus-like particles by transient transfection in HEK293 cells, suggesting that external and internal cellular mechanisms are involved (Pérez-Rubio et al., 2024).

3. Major bioreactor systems for CM

3.1. Stirred-tank bioreactors

A stirred-tank bioreactor (STR) is a relatively simple cylindrical vessel equipped with a centrally located motorized impeller for mixing cells in suspension to enable adequate nutrient supply and gas exchange (Fig. 1A). STRs are the most popular bioreactor type used to scale up mammalian cell cultivation in the manufacturing industry, mainly due to their simplicity, resilient design, and flexibility across various bioprocesses (Kuystermans and Al-Rubeai, 2015). They are compared to the other bioreactors discussed in this section in Table 1. STRs are outstanding for the dynamic cultivation of single cells or cell aggregates in suspension, or adherent cells attached to microcarriers in suspension (Olmer et al., 2012; Iworima et al., 2023; Tzimorotas et al., 2023). They are considered the standard bioreactor type for CM production (Ge et al., 2023) and have been successfully used for both cell expansion and cell differentiation (Tzimorotas et al., 2023). Laboratory-scale production was demonstrated by expanding cells in 25-mL or 100-mL STRs with and without microcarriers (Norris et al., 2022). For large-scale production, one of the biggest companies working on CM has announced the construction of 10 new bioreactors for CM with each bioreactor having a capacity of 250,000 L (GoodMeat, 2022). If realized, this would be the world's largest CM production facility.

STRs have the advantage of simplicity and adaptability (Porto de Souza Vandenberghe et al., 2024), which allows the precise control of process parameters such as temperature, pH, and agitation speed, thus enabling consistent and reproducible cell culture conditions (Fernandes-Platzgummer et al., 2023). Given the flexibility of these bioreactor types, an automated platform with well-understood bioprocess dynamics can easily be established in fed-batch or continuous mode (Bellani et al., 2020).

Despite the widespread use and effectiveness of STRs, impeller-driven mixing can generate substantial shear stress, which is detrimental to shear-sensitive cells such as those used in CM production. Computational fluid dynamics (CFD) simulations have highlighted the distribution of shear stress within the bioreactor, identifying high-shear zones near the impeller blades (Zhang et al., 2021). This stress affects cell viability, particularly that of delicate or shear-sensitive cell types (Mohseni et al., 2023). It is therefore necessary to optimize impeller design and operating conditions to minimize these adverse effects. Large scale STRs can also lead to gradients in O_2, CO_2, pH, and temperature, as well as reduced gas hold-up, all of which can be detrimental to cell health (Li et al., 2020). The use of microcarriers in STRs is also a drawback because the cells need to be detached before harvesting (Pajčin et al., 2022). This may result in alterations to cell morphology, and incomplete cell detachment reduces the product yield (Santos et al., 2023). These challenges can be addressed by the utilization of edible microcarriers, which not only allow cultured cells to remain on the carrier, but could also be exploited to enhance the flavor, color and texture of the final product (Bodiou et al., 2020; Kulus et al., 2023).

3.2. Hollow-fiber bioreactors

Hollow-fiber bioreactors (HFBs) contain a bundle of semi-permeable tubular fibers that allow nutrients and waste products to pass through the walls while retaining the cells (Fig. 1B). HFBs were developed to mimic blood vessels and allow the cultivation of both suspension and adherent cells (Schlößer and Nold, 2023). They provide a 3D microenvironment that more closely mimics the spatial distribution and interactions of cells within living organisms, providing an accurate model for the study of biological processes (Pörtner et al., 2007). This capability is particularly valuable for CM, where the creation of a tissue-like structure is necessary to mimic traditional animal products (Post, 2012; Tuomisto et al., 2022). HFBs were proven suitable for CM production when mouse C2C12 cells were cultured as spheroids in the fibers (Baba and Sankai, 2017). A HFB containing micro-anchor arrays of fibers was shown to induce the alignment of muscle cells, suggesting its suitability for the induction of skeletal muscle formation for CM production (Nie et al., 2022).

HFBs offer several practical advantages, including a compact design that supports higher cell densities than microcarriers in STRs (Tuomisto et al., 2022). The high cell density in the capillary space allows the production of large quantities of cells in a relatively small volume, making HFBs particularly suitable for applications requiring high productivity in a compact space (Javed et al., 2024). Another advantage of HBFs is that fluid flow and mixing occur primarily within the hollow fibers, away from the cells, reducing exposure to shear forces (Qin et al., 2024). These hollow fibers are designed with pores that allow small molecules and nutrients to flow into the cell compartment. The fibers are connected to a media reservoir, a gas exchanger, and a recirculation pump, creating an integrated bioreactor system (Garcia et al., 2024). In this configuration, the recirculation pump continuously moves the culture medium through the hollow fibers, providing essential nutrients and oxygen to the cells while removing CO_2 and metabolic waste (Tantakitti et al., 2023). This diffusion process is highly efficient, enabling rapid and effective nutrient uptake by the cells and promoting robust cell growth and activity. The controlled environment within HFBs ensures that cells receive a consistent supply of nutrients and oxygen, which is needed to achieve high cell densities while ensuring cell viability.

The disadvantages of HFBs include restricted scalability due to potential concentration gradients for oxygen, nutrients, and waste products within the fibers. This could greatly affect the uniformity of the final product (Tantakitti et al., 2023). Another disadvantage is that most of the materials used for fiber membranes (polysulfonate, polyethersulfonate or polyvinylidene fluoride) are inedible and may contaminate the cells during harvesting. The utilization of edible fiber materials in the HFBs (Weissenbach et al., 2022) would address this issue, and has similar advantages to the use of edible microcarriers (Fang

Table 2
Novel bioreactor concepts for CM production.

Patent number and holder	Bioreactor type	Novelty /advantage	Limitation	Cell type	Mode of operation	Reference
WO2024085898-A1 Upside Food	HFB	Pipe-based bioreactor contains substrate for cell attachment and it is rotatory to allow mixing of medium for cell cultivation	Narrow pipe geometries with high density cell growth may increase the difficulty of maintain sterile and clog-free condition	Adhesion	Batch/fed-batch	Benton and Müller-Auffermann, 2024
WO23224484-A1 Mosa Meat	STR	Able to fabricate cell-embedded hydrogel fiber in container vessel for fat cell cultivation	Precise control is required to fabricate hydrogels, and it is complicated to scale-up.	Adhesion	Batch/fed -batch	Mazari et al., 2023
WO2023211726-A1 ARK Biotech	FBB	Scalable layered scaffold-tray architecture to hold the scaffold with pulsatile flow over cells for enhanced transport	Intermittent perfusion can lead to uneven nutrient distribution across layers	Adhesion	Batch/fed-batch	Zheng et al., 2023b
WO2021148663-A1 Fraunhofer IME	FBB	Bioreactor is able to provide a mechanical stimulation to promote cell maturation in scaffold or hydrogel	Require compressible scaffold/ hydrogel to be stimulated over the batch runs	Adhesion	Batch/Fed-batch	Vogel and Schillberg, 2021
WO23211696-A1 ARK Biotech	ALB	Sparger driven air lift vertical circulation system allowing for efficient mass transfer	Require precise real-time monitoring and control to spargers and loop	Suspension	Continuously	Zheng, et al., 2023a

et al., 2024).

3.3. Rocking-motion bioreactors

Rocking-motion bioreactors (RMBs) consist of a pre-sterilized, flexible chamber, usually a plastic bag partially filled with culture medium and suspended single cells, aggregates, or cells attached to microcarriers. The chamber is placed on a rocking platform with a controlled back-and-forth motion (Fig. 1C). This rocking motion creates dynamic waves at the liquid–air interface, which enhances gas exchange and ensures thorough mixing throughout the chamber (Sharma et al., 2022). This not only promotes uniform cell growth, but also helps to keep cells and microcarriers in suspension, preventing sedimentation and ensuring an even distribution of nutrients.

RMBs were first designed as robust cell cultivation platforms that minimize shear stress, thus helping to maintain cell viability and functionality (Singh, 1999). A 1000 L scale CELBIC® RMB has been developed for biological applications (Kim et al., 2024) and the Kuhner orbital shaking bioreactor has been scaled-up to 2000 L for cell culture (Klöckner et al., 2012). These large-capacity bags offer significant advantages in terms of scalability, sterility, and operational flexibility (Djisalov et al., 2021), while reducing risks of cross-contamination and simplifying the bioprocessing workflow by eliminating the need for rigorous cleaning and sterilization procedures between batches (Ge et al., 2023; Jankovic et al., 2023). Another significant advantage of rocking motion bioreactors is the simplicity of their bag design, which may reduce bag costs. Additionally, for the successful use of single-use materials, it is essential to prioritize the recycling of these materials and, ideally, to produce the plastic bags from renewable sources.

Despite these advantages, RMBs do have some limitations. As the volume of the cultivation chamber increases, the efficiency of mixing and gas exchange decreases, affecting the uniformity of cell growth (Ge et al., 2023). Volumes exceeding 1000 L appear impractical, thus preventing further scale-up. The disposable bags increase consumables costs and any loss of integrity compromises the entire culture. Although RMBs are effective at minimizing shear stress, the precise control of these forces can be more challenging compared to other bioreactor designs (Bai et al., 2019; Bartczak et al., 2022). These factors should be carefully considered before selecting RMBs for high-density or large-scale cultures.

3.4. Bubble column and Airlift bioreactors

In a bubble column bioreactor (BCBs) gas is introduced at the base of the column, allowing gas bubbles to ascend through the liquid ensuring uniform mixing of gas and liquid. BCBs provide a high surface area for mass transfer and are simple to operate. Airlift bioreactors (ALBs) are a specific type of bubble-column reactor that uses a gas pump to inject compressed air into the bottom of a column of medium, inducing an upward flow that mixes the medium and gas, providing highly effective simultaneous oxygenation and agitation (Fig. 1D). ALBs typically operate as loop systems in which a liquid–gas mixture drives the circulation (Bartczak et al., 2022). ALBs have gained particular attention for the production of CM because the gentle mixing environment, combined with efficient oxygen transfer, helps to minimize shear stress on cells. The feasibility of a large-scale 300 m^3 ALB was recently demonstrated for the production of CM (Li et al., 2020). CFD simulations showed that the hydrodynamic and mass transfer properties of ALBs met the necessary criteria for cell cultivation. The authors also proposed a rational approach for the scaling up of CM production in ALBs.

Although the gentle mixing method reduces shear stress, one disadvantage is that it can allow cell aggregation, resulting in unwanted clumping (Cerrone et al., 2024; Sharma et al., 2022). The mixing method is also inefficient if the viscosity of the culture broth is high due to high cell numbers, making the system more difficult to scale-up than STRs. Another disadvantage of ALBs is the potential for foam formation since the agitation caused by the rising bubble in the ALB can trap air leading to the formation of foam which can affect the productivity of the bioprocess.

3.5. Fixed-bed bioreactors

Fixed-bed bioreactors (FBBs), also known as packed-bed bioreactors, typically feature a solid supporting matrix in a stationary bed for cell attachment (Fig. 1E). In contrast to other systems, where cells are either suspended directly in the medium or attached to microcarriers, FBBs require cells to be attached to the fixed bed (Lesch et al., 2021). The culture medium flows over or through the bed, enabling the diffusion of nutrients to the immobilized cells while simultaneously facilitating the removal of waste products (Cai et al., 2024).

FBBs are particularly suitable for the production of CM because they allow direct cultivation on a scaffold during cell proliferation and differentiation with low shear stress, while facilitating medium exchange (Moritz et al., 2015). In this type of fluidized-bed reactor, high-density cell cultures can be achieved by facilitating mixing through the fluidization of the medium, which eliminates the need for mechanical stirring (Post et al., 2020). Nevertheless, these systems have only been scaled up to 100 L and it remains unclear whether this level of productivity can be maintained in larger-scale vessels. Scaling out by parallelization is possible as an alternative.

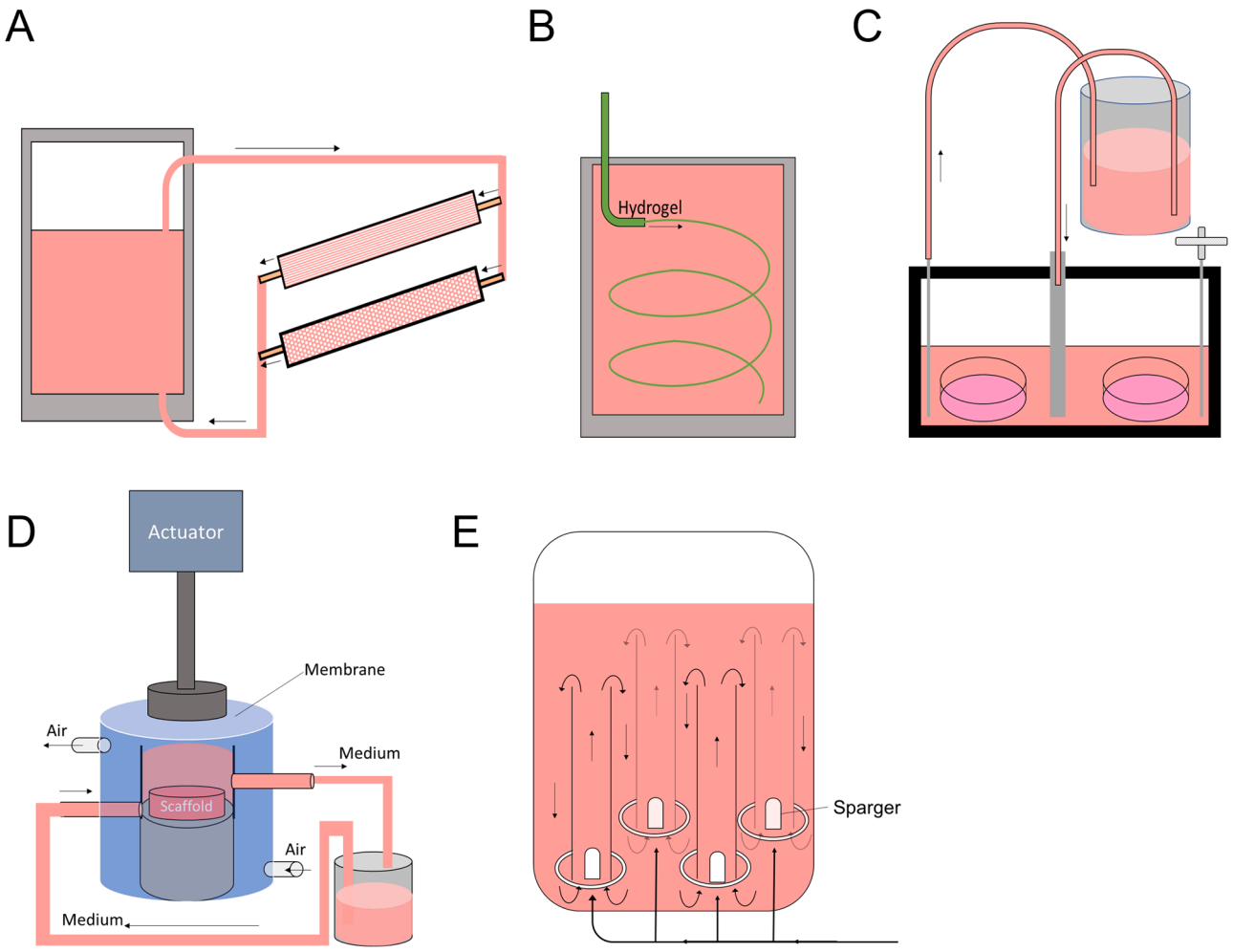

Fig. 2. Novel bioreactor concepts for CM production. (A) Pipe-based bioreactor for producing CM (Upside Foods). The pipes bioreactor contains substrates which is rotatable to allow adherent cells to attach. (B) Bioreactor for cultured fat (Mosa Meat). A vessel with an injection of hydrogel precursor fluid to fabricate a fiber for fat cell cultivation. (C) Scaffold bioreactor with scaffold trays containing scaffolds and a circulatory system to provide medium to the cells on the scaffolds (ARK Biotech). (D) A bioreactor for cultivating cells with a mechanical stimulation system to promote the cell maturation in a scaffold (Fraunhofer IME). (E) Cluster ALB with many spargers driven vertical circulation system allowing for efficient mass transfer (ARK Biotech).

Despite the high cultivation surface area of approximately 600 m^2, supporting cell densities as high as 1×10^8 cells/mL (Kurosawa et al., 2000; Meuwly et al., 2007), the harvesting of cells from FBBs can be more challenging compared to other bioreactor types (Goral et al., 2024). The substrates used as a matrix for FBBs (typically glass, polypropylene, polyurethane, or ceramics) can also introduce inedible contaminants (Pajčin et al., 2022; Pörtner et al., 2007). As with other bioreactor types, the use of edible matrices based on plant proteins or carbohydrates could help to overcome these issues, as long as such matrices remain stable during cultivation.

3.6. *Novel innovative bioreactor concepts for CM production*

Inspiration gained from the bioreactor types discussed above has sparked a wave of recent innovations for the development of novel bioreactors specifically designed for CM production. This has resulted in patents covering solutions for the scaling up of CM production as well as bioreactor designs and technologies to optimize cell growth, differentiation, and tissue formation in a controlled environment (Table 2, Fig. 2). From specialized scaffolding systems to advanced nutrient delivery mechanisms, the patented innovations will drive the development of CM from concept to commercialization.

One of these innovations is a pipe-based bioreactor (Fig. 2A) discussed in patent WO2024085898-A1 filed by the CM company Upside Food (Benton and Müller-Auffermann, 2024). This HFB variant enables the culture of adherent cells on substrates within an elongated enclosure bioreactor that is similar to the interior profile of a pipe. The substrates are arranged at nominal distances for optimal cell growth and tissue formation. In some embodiments, multiple pipe-based bioreactors are interconnected with a fluid source, enhancing the production process.

Patent WO23224484-A1 filed by Mosa Meat describes the formation of hydrogel fibers (Fig. 2B) within a vessel (Mazari et al., 2023). The method involves the injection of a hydrogel precursor fluid into a volume of cross-linking solution within a container vessel, which results in the formation of hydrogel fibers. The bioreactor is designed for the cultivation of fat, with the hydrogel fibers composed of a hydrogel precursor fluid containing fibro-adipogenic progenitors, which give rise to fat cells.

Patent WO2023211726-A1 filed by ARK Biotech also describes a scaffold bioreactor (Fig. 2C), but in this case it is a type of FBB (Zheng et al., 2023b). The aim is to support cell proliferation and/or differentiation for the production of tissues with a defined composition, structure, density, size, texture, and shape while balancing efficient nutrient and oxygen/waste exchange. The interior of the scaffold bioreactor is designed without moving parts, maximizing space utilization and reducing production costs, while making the vessel easy to clean and sterilize. The scalable design can be tailored to meet specific needs in process development, pilot production, and industrial operations,

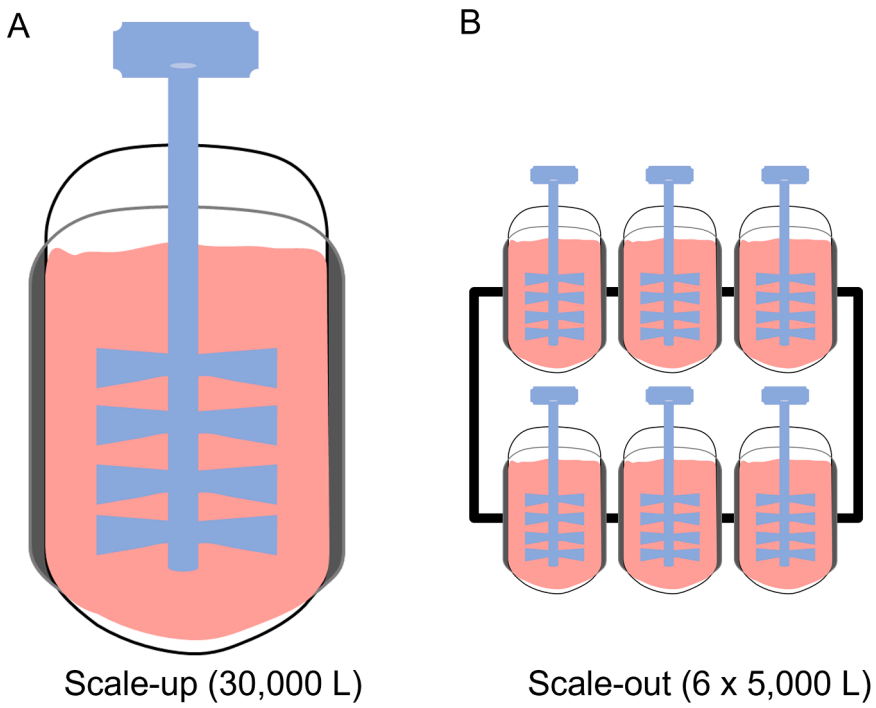

Fig. 3. Schematic demonstrating large-scale CM production of by scaling- up using a single large bioreactor (A) and (B) by using multiple small bioreactors in parallel (scaling-out or parallelization).

providing a straightforward scale-up process that minimizes risk and accelerates the industrialization of whole meat cuts.

Patent WO2021148663-A1 filed by the Fraunhofer Society (Vogel and Schillberg, 2021) describes a bioreactor comprising a vessel that contains an elastic scaffold to which animal cells can attach (Fig. 2D). A membrane closes the vessel from above and can be moved up and down by a piston. This compresses and relaxes the scaffold, ensuring the supply of nutrient medium to the cells and inducing their differentiation via mechanical stimuli. This process can be scaled out by parallelizing the bioreactors, resulting in the production of a finished piece of meat in each vessel.

Finally, ARK Biotech filed another patent (WO23211696-A1) describing a novel, vertically oriented ALB that features multiple spargers and vertical circulation loops (Zheng et al., 2023a). Each vertical circulation loop consists of a sparging region and a return region, with the loops being connected (Fig. 2E). The bioreactor controller manages the spargers to ensure consistent mass transfer across all loops, making it ideal for precise oxygen and nutrient distribution in large-scale cultures.

The novel bioreactor concepts utilize small bioreactors that operate with low volumes (Fig. 2), which may not initially appear suitable for large-scale CM production. However, the CM production process can be effectively scaled out by running multiple of these novel small bioreactors in parallel (Fig. 3). This approach usually demands less capital investment compared to larger units, facilitating market entry for companies, particularly startups.

4. Stimuli for cell differentiation

4.1. Mechanical stimulation

4.1.1. Culture substrate stiffness

Optimizing the differentiation process helps to improve the quality and acceptability of the CM product because this determines the texture, flavor and nutritional profile (Martins et al., 2024; Shaikh et al., 2021). The stiffness of the material on which cells grow has a significant impact on cell differentiation by modulating the mechanical environment. When cells are exposed to mechanical stress, such as that induced by the stiffness of the substrate or applied forces, they respond by activating signaling pathways that determine their fate. For example, mechanical stress gradients can control the differentiation of stem cells into different lineages, such as mesodermal and ectodermal, by altering cytoskeletal dynamics and gene expression patterns (Muncie et al., 2020). In addition, the material properties of the cells themselves, such as their stiffness, play a decisive role in how they react to mechanical forces. Softer cells are more sensitive to mechanical stress, which can boost proliferation and differentiation, whereas stiffer cells can resist such changes. This interplay between material tension and cellular responses is crucial for the optimization of tissue engineering strategies. This also applies to the production of CM, where the precise control of cell differentiation is required to achieve the desired texture and quality of the final product (Bergert et al., 2019; Chowdhury et al., 2010).

Mechanical stimuli regulate the proliferation and differentiation of muscle stem cells and adipogenic progenitor cells via different signaling pathways (Lee et al., 2024; Messmer et al., 2023). In muscle cells, the application of mechanical forces in bioreactors can control the differentiation of myoblasts into mature myotubes and myofibers, which determine the texture of CM (Bomkamp et al., 2023). The strength and type of mechanical force synergizes with growth factors to determine the fate of muscle cells (Post, 2012). Material tension also influences adipogenic differentiation by modulating the mechanical environment. The stiffness of the substrate and applied forces can activate signaling pathways that determine the fate of adipogenic progenitors, with softer materials promoting proliferation and differentiation (Lee et al., 2024).

4.1.2. Shear stress

Shear stress is detrimental to sensitive cells, but can provide an important mechanical stimulus that influences differentiation. It can activate intracellular signaling pathways that regulate the differentiation of stem cells into specific cell lineages, such as muscle cells. Exposing cultured muscle cells to shear stress promotes the fusion of myoblasts and the formation of myotubes, which contribute to the texture of CM products. Liquid shear stress can activate mechanosensitive signaling pathways such as the PI3K/Akt and MAPK/ERK pathways,

triggering the expression of muscle-specific genes and promoting cell alignment and organization (Haroon et al., 2022). The application of shear stress mimics the mechanical forces *in vivo*, providing a physiologically relevant environment that can improve cell maturation and functionality. By optimizing shear stress conditions in bioreactors, the efficiency and quality of cell differentiation can be improved, ultimately contributing to the production of high-quality CM products that are similar in texture and composition to conventional meat (Huang et al., 2021; Kim et al., 2014; Lemke and Schnorrer, 2017; Park et al., 2013; Sunadome et al., 2023; Vining and Mooney, 2017; Yourek et al., 2010).

4.1.3. Topographical cues from scaffolds

Scaffolds with particular surface structures, such as aligned fibers or grooves, can promote the alignment and elongation of muscle cells, the fusion of myoblasts, and the formation of functional myofibers. For example, skeletal muscle cells cultured on aligned scaffolds differentiate more quickly and express muscle-specific markers at higher levels than cells on flat or randomly structured surfaces, allowing for better tissue organization and functionality (Chi et al., 2022; Metavarayuth et al., 2016). The topographic features of scaffolds may also control the behavior of adipocyte precursors and promote their maturation into functional adipocytes. Specific surface patterns can improve cell adhesion and lipid accumulation in adipose-derived stem cells, contributing to the overall quality of cultured adipose tissue (Alarcin et al., 2021; Lu et al., 2022). By adapting the topography of the scaffold to mimic the natural ECM, we can create optimal conditions for the differentiation of both muscle and fat cells.

4.2. Electrical stimulation

Electrical stimulation regulates muscle cell behavior *in vitro* and can be used to improve skeletal muscle cell alignment and differentiation by mimicking the electrical signals naturally present in muscle tissue, promoting fusion and the formation of mature myofibers (Ahadian et al., 2013; Je et al., 2019; Jun et al., 2009; Nagamine et al., 2018; Park et al., 2008). Short-term (several minutes) and low-intensity (0.06~6 V/cm) stimulation can promote the differentiation of muscle progenitor cells into mature myotubes via the formation of embryoid bodies. Electrical stimulation also regulates the interactions between the cells and the ECM. It can trigger differentiation by inducing intracellular Ca^{2+} oscillations that cause the development of mature sarcomere structures (Nagamine et al., 2018). The application of specific electrical pulse patterns can activate intracellular signaling pathways, such as the PI3K/Akt and MAPK/ERK pathways, which are necessary for muscle cell maturation and functionality (Nagamine et al., 2018; Nikolić and Aas, 2019; Thelen et al., 1997). A suitable electrode material, as well as optimal amplitude and frequency, are needed to induce the development of muscle tissue without cell damage (Nikolić and Aas, 2019). By applying appropriate stimuli, muscle progenitor cells can be efficiently differentiated into aligned, contractile myotubes to form biomimetic muscle tissues (Ahadian et al., 2013; Je et al., 2019). The combination of electrical stimulation and biocompatible conductive scaffolds can further enhance the benefits of muscle tissue engineering.

4.3. Chemical stimulation

4.3.1. Nutrient composition in cell culture media

Glucose, as a primary energy source, is essential for both muscle and adipocyte differentiation, and the glucose concentration in cell culture media can influence the expression of myogenic and adipogenic markers. For example, high glucose concentrations (25 mM) have been associated with increased myoblast differentiation and myotube formation compared to physiological glucose concentrations (5.5 mM) in C2C12 myoblasts (Lee et al., 2024). High glucose levels can also promote lipid accumulation and the expression of adipogenic transcription factors such as PPARγ and C/EBPα (Sorisky, 2017).

Amino acids, especially glutamine, are also necessary for the differentiation of muscle and fat cells. Glutamine serves as an energy source and is required for the synthesis of proteins and nucleic acids. The supplementation of cell culture media with L-glutamine or stable dipeptide forms such as alanyl-glutamine (GlutaMAX) or glycyl-glutamine improves the proliferation and differentiation of myoblasts and adipocytes (Golikov et al., 2022; Waldemer-Streyer et al., 2022). In addition, the presence of essential amino acids, such as leucine and arginine, is associated with improved muscle cell differentiation and the regulation of adipogenesis (Kim et al., 2014)(Burd et al., 2019).

4.3.2. Growth factors and cytokines

Insulin-like growth factors (IGFs) play a decisive role in muscle development and regeneration. IGF-1 and IGF-2 are secreted by muscle cells and act as autocrine factors to promote the proliferation and differentiation of myoblasts and stimulate the expression of myogenic markers such as myogenin (Ahmad et al., 2020; Bach, 2004). IGF-1 also promotes mesenchymal stem cells to enter the adipogenic lineage and facilitates the proliferation of pre-adipocytes and their differentiation into mature adipocytes (Al-Mansoori et al., 2022; Hu et al., 2015). Cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) are produced by muscle cells and can modulate their differentiation. For example, IL-6 promotes myoblast differentiation under certain conditions, whereas TNF-α can have positive or negative effects depending on timing and concentration, influencing inflammation and muscle regeneration (Gleeson et al., 2011; Waldemer-Streyer et al., 2022). TNF-α inhibits lipoprotein lipase activity and promotes apoptosis, but it also plays a complex modulatory role in adipogenesis by affecting the expression of PPARγ (Al-Mansoori et al., 2022; Hube and Hauner, 1999; Zhao et al., 2013). IL-6 has both pro-inflammatory and anti-inflammatory effects and contributes to the regulation of fat cell differentiation and activity (Han et al., 2020). Interactions between these growth factors and cytokines create a complex regulatory network that fine-tunes the differentiation of muscle cells and ultimately influences muscle development and repair, as well as promoting or inhibiting adipocyte differentiation and influencing adipose tissue function and metabolic health.

4.4. Environmental stimulation

4.4.1. ECM components

Specific ECM proteins such as collagen type I, laminin and fibronectin, are essential for the adhesion, proliferation and differentiation of myoblasts into mature myotubes (Gillies and Lieber, 2011). Type I collagen provides tensile strength, whereas laminin promotes myoblast attachment and activates signaling pathways that improve myogenic differentiation. Fibronectin facilitates cell adhesion and migration, and the formation of organized muscle tissue (Ahmad et al., 2023; Thorsteinsdottir et al., 2011). Similarly, ECM components such as collagen type IV, hyaluronic acid, and vitronectin influence adipogenic differentiation (Clevenger et al., 2016; Sillat et al., 2012; Zhu et al., 2019). Collagen type IV is an important component of the basal lamina, which supports the structure and function of adipocytes (Sillat et al., 2012). Hyaluronic acid, a glycosaminoglycan, plays a role in cell signaling and can enhance the expression of important adipogenic transcription factors such as PPARγ and C/EBPα, thus increasing lipid accumulation and promoting the functional maturation of adipocytes (Zhu et al., 2019). Vitronectin also supports the adhesion of fat cells and promotes their differentiation (Clevenger et al., 2016; Ruiz-Ojeda et al., 2019).

5. Large-scale strategies for CM production

5.1. Scaling up by transfer to a larger bioreactor

The typical scale-up strategy for CM production is to increase the working volume of a single bioreactor (Fig. 3A), allowing the production

of more cells (Stephens et al., 2018). This approach leverages the principle that increasing the size of a bioreactor can theoretically lead to a proportionate increase in output, assuming that the bioprocess parameters can be maintained effectively at a larger scale (Humbird, 2021). However, small-scale and large-scale bioreactors differ in terms of mixing efficiency, oxygen/nutrient transfer and heat distribution, because these do not scale linearly with size (Bellani et al., 2020). One of the primary issues is maintaining a uniform environment within the bioreactor, which becomes increasingly difficult as the size increases. Specifically, stirring times increase disproportionately during scale-up due to the square-cube law, making it impossible to maintain the mixing efficiency observed in small-scale processes. In smaller bioreactors, efficient stirring ensures a uniform distribution of nutrients, gases, and cells, leading to consistent cell growth and metabolism. But in larger bioreactors, longer mixing times and less efficient mixing lead to the formation of local concentration gradients that cause cells in different regions of the bioreactor to experience varying environmental conditions, inconsistent growth rates and fluctuating metabolic activities. Mixing quality and shear forces are not proportional to each other, and not all parameters can be kept constant during scale-up. It is important that vigorous mixing in large-scale reactors does not cause excessive shear stress.

Fed-batch operations are standard practice in industrial biotechnology, and concentrated feedstock is usually dripped into the bioreactor from the top (Bylund et al., 1998). This can create local concentration gradients due to inefficient stirring. Cells near the feed inlet are exposed to higher concentrations of nutrients and possibly osmotic stress, whereas cells farther away may experience nutrient limitations. These local variations in nutrient concentrations cause cells to behave differently within these gradients, leading to heterogeneity in the culture, suboptimal growth, lower productivity, and inconsistent product quality (Bylund et al., 1998). It is important to specify the distinction between soluble products in current industrial applications and biomass as the end product in cellular agriculture applications because there is a larger knowledge gap in scale-up strategies where biomass is the primary product.

Inadequate mixing can also create oxygen gradients and foaming issues (Bellani et al., 2020; Pörtner et al., 2007). Oxygen is only sparingly soluble in aqueous solutions, and the increased demand for oxygen in larger volumes means that oxygen transfer can become a critical limiting factor. Oxygen transfer depends on the gas–liquid interfacial area, oxygen solubility, and the oxygen transfer coefficient (kLa). In large bioreactors, it becomes difficult to maintain sufficient oxygen transfer rates without causing excessive shear stress due to the higher agitation or sparging rates.

A further technical challenge is the scale-dependent changes in hydrodynamics within bioreactors (Zhang et al., 2021). In smaller bioreactors, the flow patterns are more predictable, and cells experience relatively consistent shear forces (Zhang et al., 2021). In larger bioreactors, the distribution of these forces can become uneven due to complex flow dynamics, leading to regions within the bioreactor where cells may be subjected to either excessive shear stress or insufficient mixing (Zhang et al., 2021). Excessive shear stress can damage cells, especially shear-sensitive mammalian cells used in CM production, while insufficient mixing can lead to poor mass transfer and nutrient delivery. This inconsistency can result in variable product quality and yield (Zhang et al., 2021).

Economies of scale in CM production can be achieved by producing larger quantities of cells in single bioreactors, lowering production unit costs and facilitating the market entry of CM products (Humbird, 2021). Successful scale-up has been demonstrated in pharmaceutical bioreactors, such as the production of monoclonal antibodies, where working volumes of up to 20,000 L are not unusual (Seamans et al., 2008). The success of antibody production offers valuable insights for the CM industry. In the case of antibodies, advances were achieved by developing optimized nutrient delivery systems and more efficient bioreactor designs, facilitating high yields in unstructured cell environments. However, muscle cell culture presents unique challenges. Unlike antibody production, which relies on cells in suspension, muscle cells require a structured environment, such as scaffolds, to grow in a manner that mimics native tissues. These scaffolds must integrate nutrient delivery and oxygenation while maintaining cell viability. Additionally, the sensitivity of mammalian cells to shear stress becomes critical when scaling up production, where mixing efficiency, nutrient distribution, and maintaining structural integrity can come into conflict. The largest published scale of unstructured cell culture so far differs significantly from the structured approaches needed for CM, where the geometry of the scaffold and the integration of mechanical cues play pivotal roles in mimicking the texture and function of muscle tissue. It is important to acknowledge the limits of scaling bioreactors beyond a specific size. Given that the biomass itself is the product of CM production, there is a practical upper limit for process control. Rheological properties, such as increased viscosity at high cell densities, can also negatively affect mass transfer and mixing efficiency. Viscosity sharply increases beyond a specific cell density, imposing further restrictions on bioreactor scalability (Humbird, 2021).

Scaling up production using a single large bioreactor entails substantial financial investment (Negulescu et al., 2023), which poses a significant risk, particularly in the absence of established market acceptance. This investment not only covers the cost of the bioreactor itself but also extends to operational expenses, maintenance, and the necessary infrastructure.

5.2. *Scaling out by the parallel operation of multiple bioreactors*

The scale-out approach, or parallelization, increases production capacity by operating multiple smaller bioreactors in parallel (Fig. 3B) (Gome et al., 2024; Santos et al., 2023; Stephens et al., 2018). Each bioreactor operates under identical conditions, and the total production volume is achieved by aggregating the outputs from all the bioreactors (Stephens et al., 2018). This method avoids the complexities of scaling up a single large bioreactor by maintaining the well-understood, optimized conditions of smaller bioreactors. One of the primary technical advantages of the scale-out strategy is the ability to maintain consistent and controlled conditions across all bioreactors. Because each bioreactor operates independently, the risk of process failure is distributed, and the impact of any single bioreactor failure on overall production is minimized. This modular approach also allows for greater flexibility in production: capacity can be scaled incrementally by adding more bioreactors as demand increases without needing significant upfront capital investments. However, the scale-out strategy lacks the economies of scale that can be achieved by scale-up (Fish et al., 2020). This is because the operational costs associated with scale-out are higher due to the need for more equipment, reagents, space, and labor (Seamans et al., 2008). Managing multiple bioreactors requires automation and control systems to ensure each bioreactor operates under identical conditions. Multiple devices are often used in parallelized bioreactor systems to generate a quasi-continuous output. Identifying the optimal configuration for CM production, such as the number of bioreactors, timing of cultivation cycles, and bioreactor sizes, still requires further research. Smart bioreactors equipped with real-time monitoring and feedback systems can ensure that conditions are consistently maintained across all units (Djisalov et al., 2021) and smart feeding strategies (Hubalek et al., 2022). As the industry continues to evolve, hybrid approaches that combine the benefits of both scale-up and scale-out may emerge as the most effective solution for large-scale production.

6. Conclusions

In this review article, we have considered various aspects of CM production in bioreactors that contribute to the success of the process and the quality of the final product. The most important elements are the

Table 3
Key performance indicators (KPIs) for the design of bioreactors for CM production.

KPI	Description
Bioreactor type	The choice of bioreactor design influences the mass transfer, mixing and shear stress. It also determines the operating volume and scale up potential. Therefore, the bioreactor type must be considered for CM production and scale-up.
Mode of operation	For large-scale CM production, the fed-batch or continuous supply of media to the cells is preferred for efficient cell proliferation.
Fluid dynamics and agitation systems	Essential to limit shear stress, and to control the temperature and gas gradient.
Proliferation/ differentiation	The bioreactor should support a high cell density, thus ensuring robust cell proliferation and differentiation. Moreover, the cultivation process should be conducted in a manner that maintains cell viability and minimizes cell death.
Ease of operation	The ease of operation ensures that the processes of feeding, extraction, and control are straightforward and efficient. This allows for the smooth handling of the system and minimal downtime while maintaining precise control over the bioprocess.
High yield and reproducibility of results	Achieving high yield and reproducibility is critical to ensure that the system consistently supports optimal cell growth and production efficiency over multiple cycles while maintaining product quality.
Ease and cost of maintenance	The bioreactor should be designed to have minimal maintenance requirements and to be cost-effective. It is constructed in such a way that the components can be accessed with ease and that there is minimal downtime. This guarantees that routine maintenance and repairs can be carried out with minimal operational disruption and reduced long-term maintenance costs.
Durability	The bioreactor should be able to withstand continuous use over extended periods, maintaining structural integrity and performance under various operating conditions, including pressure/ temperature fluctuations, and chemical exposure.
Operating volume and up scaling	The bioreactor should be designed in such a way that it can be readily scaled up without altering cell growth.
Cleaning and sterilization	Cleaning and sterilization of the bioreactor should be easy, inexpensive and compliant with the regulations.
Sterility risks	A sterile environment is essential to prevent contamination from unwanted microorganisms, which can compromise the entire CM production process.
Process monitors and control	Effective process monitoring and control are essential, allowing for real-time adjustments to parameters such as temperature, pH, oxygen levels, and nutrient supply to maintain optimal conditions and ensure consistent product quality throughout the bioprocess.
Sustainability	The bioreactor design should minimize the consumption of media, energy, and water during the process.
Material properties and equipment selection	Appropriate materials and equipment ensure optimal performance, durability, and compatibility of the bioreactor with the bioprocess. The materials must demonstrate chemical resistance and mechanical strength. The equipment must be chosen to enhance process control, scalability, and efficiency, in alignment with the specific demands of the cell culture environment.

bioreactor design (which must be optimized not only for the recovery of biomass as the final product, but properly structured biomass that resembles livestock-derived meat in texture, taste, and color), the process parameters (pH, oxygen, CO_2, temperature and cell density), and the specialized methods that are used to promote cell proliferation and differentiation using mechanical, electrical, (bio)chemical and environmental stimulation, or combinations thereof. We have also considered the two main strategies for increasing the scale of production, namely scaling up and scaling out. The conclusions from this article can be synthesized as a set of key performance indicators (KPIs) influencing bioreactor design and process optimization for the production of CM, which are summarized in Table 3. These KPIs should be considered when developing or optimizing processes for the production of CM, and are particularly relevant in an industrial context when scaling from a laboratory process.

Ethical statement – studies in humans and animals

No animal and human studies were conducted to prepare this article.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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