Trends in Food Science & Technology 97 (2020) 443–450

Contents lists available at ScienceDirect

Trends in Food Science & Technology

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

Challenges and possibilities for bio-manufacturing cultured meat

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ARTICLE INFO

Keywords:
Cultured meat
Tissue engineering
Cell culture
Serum-free culture
Bioreactor
Synthetic biology

ABSTRACT

Background: Cultured meat has emerged as a breakthrough technology for the global food industry, which was considered as a potential solution to mitigate serious environmental, sustainability, global public health, and animal welfare concerns in the near future. Although there is promise that cultured meat can supplement or even replace conventional meat, many challenges still need to be resolved in the early stages.

Scope and approach: In this review, we focused on the characteristics of cultured meat and summarized the current technological challenges and their possible solutions based on tissue and bioreactor engineering, food science, and material science for preparing stem cells, optimizing culture conditions, and developing cost-effective culture media, bioreactor designs, and food processing systems.

Key findings and conclusions: With rapid progress in tissue and bioreactor engineering, new technologies for culturing meat have been developed and significant progress has been made in recent years. However, as research on cultured meat is intrinsically complex, it is necessary to encourage the integration of multidisciplinary research in this field in the future. We propose some innovative approaches, such as the applications of synthetic biology and bioreactor engineering. These strategies will be helpful to scale-up cultured meat in future applications.

1. Introduction

The term “cultured meat”, also described as *in vitro*, synthetic, or lab-grown meat, refers to meat produced in a bioreactor with tissue engineering technology (Bhat & Fayaz, 2011; Stephens et al., 2018; Tiberius, Borning, & Seeler, 2019). In contrast to conventional meat, cultured meat promises to address financial concerns, animal welfare ethics, resource shortages, and public health issues (Bhat, Kumar, & Bhat, 2017; Stephens et al., 2018).

The idea of cultured meat as an alternative to conventional meat was originally envisioned by Frederick Edwin Smith and Winston Churchill in 1930s (Arshad et al., 2017). A laboratory investigation of cultured meat was first designed by the National Aeronautics and Space Administration (NASA) in the early 2000s, with the aim of growing myoblasts in suspension culture as a sustainable supply system for long-term space flights and space stations (Benjaminson, Gilchriest, & Lorenz, 2002; Wolfson, 2002).

In recent years, advances of tissue engineering in regenerative medicine have helped scientists obtain muscle tissue from a section of living samples with cell cultures, paving the way for *in vitro* meat production (Arshad et al., 2017; Bhat & Bhat, 2011b; Vein, 2006). It is practical to use skeletal muscle tissue engineering, stem cells, cell co-cultures, and tissue culture methods for *in vitro* meat culturing, all of which avoid livestock problems such as animal harm and environmental pollution. The well-known prototype of *in vitro* meat patty was developed in 2013 by pharmacologist Dr. Mark Post and it looked similar to a conventional meat. This event raised public expectations for cultured meat as a meat substitute (O'Riordan, Fotopoulou, & Stephens, 2017; Post, 2014). At the time, however, it took about three months and more than $330,000 to grow the five-ounce meat patty in a laboratory. Indeed, cultured meat production is still in its early stages, and limiting factors of high costs and inefficient technology remain, blocking its application and commercialization (Post, 2012; Stephens et al., 2018). To adopt cultured meat, it is important to mimic the required key

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https://doi.org/10.1016/j.tifs.2020.01.026
Received 17 September 2019; Received in revised form 12 January 2020; Accepted 19 January 2020
Available online 23 January 2020
0924-2244/

qualities of conventional meat, such as physical appearance, smell, texture, and taste. This review focuses on the advantages and development of cultured meat, discusses the technical challenges, and suggests potential strategies for addressing issues in cultured meat production.

2. Benefits of cultured meat

2.1. Relieving animal suffering

According to the recently adjusted forecast by the Food and Agriculture Organization (FAO), the demand for meat will increase by up to 70% by 2050, which will be an immense challenge for the livestock system (Gerber et al., 2013). As an alternative, cultured meat production systems promise to supply *in vitro* meat/protein to meet global demands, which would mitigate the slaughter of millions of food animals (Bhat & Bhat, 2011a; Webster, 2002). In theory, each parent cell involved in cultured meat production can proliferate many number of times. The number of animals required for tissue samples are thus lesser than for conventional meat production at orders of magnitude, which might provide a promising approach to relieve animal suffering.

2.2. Health and safety

The livestock systems for conventional meat production bring risk of animal disease, epidemics, and antibiotic misuse (Bhat & Bhat, 2011a; Webster, 2002). Instead, cultured meat will use safe and moderate concentrations of preservatives such as sodium benzoate to protect the growing meat from microbes (Seman, Quickert, Borger, & Meyer, 2008). Moreover, there are online monitoring systems to analyze the quality of cultured meat and provide food safety guarantee technology with a low chance of bacterial contamination during the production process. The quality of cultured meat can be optimized through controlled culture system and post-processing such as the composition proportion, nutrient content, taste, and flavor (Bhat & Bhat, 2011a).

2.3. Sustainability and environment

In traditional livestock, only 5%–25% of the animal is processed as edible meat resulting in a low conversion rate for conventional meat production (Alexander, 2011; Bhat & Bhat, 2011a). It bring a series of problems with a considerable portion of greenhouse gas emissions, land usage, and water and energy consumption (Bellarby et al., 2013; FAO, 2006; Gerber et al., 2013). Compared to most conventionally produced European meat livestock system, it has been indicated that cultured meat involves approximately 78%–96% lower greenhouse gas emissions, 99% lower land use, and 82%–96% lower water use (Mattick, Landis, Allenby, & Genovese, 2015; Tuomisto & Teixeira de Mattos, 2011). Cultured meat is potentially a sustainable and eco-friendly means of producing meat, as it can be obtained efficiently without the need to develop other supporting tissues and functional structures such as skeletal and digestive systems, once the technology is advanced adequately. Despite the early stage of development with technical challenges and consumer acceptance, such as lacking cost-effective and resource-efficient methods for scaling up, it is speculated that the overall energy balance will tip in favor of cultured meat when indirect costs and environmental benefits are taken into account (Bhat, Kumar, & Fayaz, 2015; Tuomisto, 2019). In general, cultured meat is potentially a sustainable and eco-friendly means of producing meat to relieve stress from an increasing population and fulfill the demand for meat.

3. Technical challenges to producing cultured meat

3.1. Cell resources

Animal tissue culture technology originally began in the 1990s and was used to study cell proliferation and metabolism (Ebeling, 1914). At present, animal tissue engineering research is largely focused on biology and medicine, such as regenerative medicine, drug development, and toxicology research. Cultured meat may not require strict regulations as those required for cell cultures in medical research as the purity of raw materials needed for cultured meat is not as high as they are for biomedical applications. Therefore, it is important to develop efficient, safe, and large-scale production of cultured meat to reduce the production costs effectively (Arshad et al., 2017).

One of the challenges to cultured meat production is the selection of an appropriate cell source for the animal tissue culture (Post, 2012). The main challenge in tissue culture for cultured meat is obtaining sufficiently large number of homogeneous starter cells to conduct effective proliferation and differentiation. Over the past decades, several stem cell types have been identified, and the related technology has advanced considerably. At present, there are several cell sources applied to tissue engineering. One source is the original tissues or cell lines. Mutations are then induced through genetic engineering or chemical methods, resulting in unlimited cell proliferation (Ramboer et al., 2014). These continuously proliferating cells can reduce the dependence on fresh tissue samples and increase the rate of cell proliferation and differentiation. However, there are also some problems associated with cell lines derived from stem cells including genetic instability and phenotypic drift. Cell line genetic and phenotypic instability, together with misidentification and contamination with microorganisms, are one of the several problems that continue to affect cell culture (Geraghty et al., 2014). Another source is stem cells isolated from tissues, such as embryonic stem cells, muscle stem cells, and mesenchymal stem cells (Fig. 1) (Stern-Straeter et al., 2014). Muscle stem cells (satellite cells) (Mauro, 1961) are the most widely used in research on cultured meat due to their potential for differentiation. These cells can differentiate into specific cells through chemical, biological, or mechanical stimulation during the process of proliferation (Post, 2012). Although theoretically, various stem cell lines can grow indefinitely after they are established, the accumulation of cell mutations during the proliferation process often affects the amplification ability of tissue cultures, leading to the termination of cell aging (Amit et al., 2000).

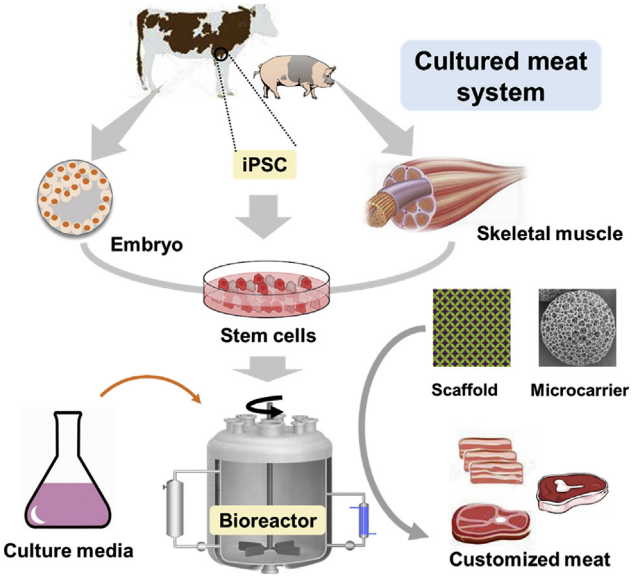

Fig. 1. Production flow chart of cultured meat. Stem cells obtained from muscle tissue, embryos, or induced somatic cells are first expanded and then induced for differentiation into muscle cells. These cells are further grown in a bioreactor to increase their number. Scaffolds or microcarriers are then introduced to grow these cells into specific muscle fibers and larger tissues. Figure elements adapted from published paper Tuomisto HL (2019) *EMBO Rep. 20(1): e47395.*

In order to further expand cell sources, research on transforming somatic cells into induced pluripotent stem cells (iPSC) has also received attention. iPSCs are differentiated cells and rendered pluripotent by stable transfection with a set of specific transcription factors driving embryonic gene expression programs in the cell (K. Takahashi & Yamanaka, 2006). Stem cells can thus be effectively prepared to achieve mass cell proliferation (Bogliotti et al., 2018; Wu & Hochedlinger, 2011). Recently, different strategies have been proposed for generating safe iPSCs without any genomic modification. These pertain to the type of somatic cells for reprogramming, variations of the reprogramming genes, and reprogramming methods. This is a relatively promising but challenging technology for cultured meat production derived from iPSCs.

3.2. Proliferation and differentiation

The development of stem cells and tissue engineering provides the possibility of large-scale cultured meat production (Cravero et al., 2015; L.; Zhao et al., 2017). Cultured meat requires a large number of differentiated muscle cells to form tissues. Studies have indicated that it is feasible to maintain healthy cells by providing fresh nutrients, while cell passaging or splitting is required to maintain cells in exponential growth (Masters & Stacey, 2007). However, most cells have a limited capacity for division, known as the Hayflick limit, which limits large-scale cultures of muscle tissue in a laboratory. There is another effective way to enhance proliferation by increasing the regenerative potential of stem cells. For example, the Hayflick limit is determined by the telomere length, which is a repeating sequence rich in guanine at the end of chromosomes. The telomeres shorten with each round of replication, affecting the ability of the cell to proliferate. Telomerase, a ribozyme that lengthens telomeres, is found in anti-aging cell lines. Therefore, regulating the expression of or exogenous addition of telomerase can effectively improve cell regeneration potential, which is conducive to the large-scale, stable, and rapid proliferation of animal cells (Shay & Wright, 2000).

A patterned co-culture is a promising technique used to investigate cell-to-cell communication and tissue engineering (Yamazoe, Ichikawa, Hagihara, & Iwasaki, 2016). To improve the quality of cultured meat, co-culture with other cells such as adipocytes should also be considered, which could improve the texture, structure, and flavor of artificial meat (Edelman, McFarland, Mironov, & Matheny, 2005; Hocquette et al., 2010). However, due to the difference in growth rate, co-culturing involves the risk of obtaining disproportionate number of different cell types. Researchers have attempted to precisely control the direction and nodes of stem cell proliferation and differentiation to achieve customized production of artificial meat with varying fat content. For example, in cattle, it is known that vitamin A deficiency is associated with an elevated intramuscular fat content (Kruk et al., 2018).

3.3. Serum-free culture media

Serum-based media have been widely used to grow animal cells *in vitro*, providing high growth-promoting activity for a range of mammalian cell lines (M. Takahashi, Makino, Kikkawa, & Osumi, 2014). The serum contains attachment factors, micronutrients, trace elements, growth factors, hormones, and protective elements that promote rapid cell growth, but also bring the risk of contamination with viruses or prions (Park et al., 2013). It is crucial to employ a low cost and safe medium for tissue engineering and scaling up cultured meat (Bjare, 1992; Leong et al., 2017; Warner, 2019).

During the last few decades, a number of serum-free medium formulations have been reported for mammalian and insect cell lines as well as for primary cultures (Bjare, 1992; Brunner et al., 2010; Tan et al., 2015). A serum-free medium usually consists of basal medium and medium supplements. The basal medium generally comprises of amino acids, vitamins, glucose, and inorganic salts, which are essential factors in cell growth and metabolism. Chemical components or growth factors could be added to the serum-free medium as supplements (Brunner et al., 2010). Generally, supplemental factors can be divided into necessary and special factors. Necessary factors are required for all cell lines to grow in serum-free medium, including transferrin, insulin, etc. Special factors include adherent factors, binding proteins, and hormones. However, the switch to serum-free media still demands a time-consuming literature survey and a manufacturer search for appropriate medium formulations. Compared to serum-based media, current serum-free media show poorer performance in growth promotion (Miki & Takagi, 2015). Although it is challenging to identify and substitute all the functional components in sera (Brunner et al., 2010), computer-aided design and synthetic biology have been adopted as efficient approaches to building chemically-defined media (Tan et al., 2015). Brunner et al. developed an interactive serum-free media online database and specified the cell lines adapted to serum-free media, in which search terms such as species, organ, tissue, and cell type can be used (Brunner et al., 2010). There have been gradual improvements with the addition of key ingredients and adapting cells to serum-free media by involving a systematic approach to replace the serum gradually by substituting with essential nutrients or growth factors (Aswad, Jalabert, & Rome, 2016), which can facilitate large-scale production of cultured meat under safe conditions.

3.4. Bioreactors

Among the reasons that large-scale production has yet to be seen are the difficulties associated with reactors and the process scaling up of cultured meat (Verbruggen, Luining, van Essen, & Post, 2018). In the biotech industry, it is generally recognized that there is an inverse relationship between a product's market size and its selling price. As of today, most products derived from mammalian cell cultures are in the high-value, low-volume territory, e.g., therapeutics and pharmaceuticals, whilst products at the other end of the spectrum, namely, food additives and animal feed, are produced by microbial fermentation (Oosterhuis, 2018). Commercially available production-scale bioreactors for cell cultures are typically 1–2 m^3 in working volume, although larger vessels up to 10–20 m^3 can be custom built (Flickinger, Hu, Zhou, Zhou, & Zhong, 2010). Still, these are much smaller than microbial reactors, which can be 200–2000 m^3. There are many reasons that smaller reactors might be preferred for cell culturing. For instance, multiple smaller units offer greater flexibility to adapt a plant's throughput and product portfolio to market fluctuations; they also offer easier damage control in the event of contamination. Perhaps the most important factor is that the fixed capital expenditure associated with those reactors only accounts for a small fraction of the total production cost. Consequently, the size of individual reactors does not significantly affect the producer's profitability. Unfortunately, for cultured meat as a bulk commodity to compete effectively with its agricultural counterparts, conventional wisdom suggests that the reactor and process need to be up-scaled by two orders of magnitude, in which case serious engineering challenges need to be addressed. It is pertinent to examine these challenges in the context of the type of reactors that can be used for large-scale cultured meat production, as some of the challenges are reactor-specific.

3.5. Scaffolding with direct perfusion

The popularity of perfusion reactors with 3D scaffolding in tissue engineering has convinced several researchers that it is the best technique for cultured meat production (Datar & Betti, 2010; Specht, Welch, Rees Clayton, & Lagally, 2018). Indeed, if the ultimate form of cultured meat is a highly structured product that mimics large cuts rather than ground meat—perhaps even completed with co-cultured red blood cells or even blood vessels—3D scaffolding does seem to be a reasonable choice. However, owing to the micro-porous structure of the scaffold

material, media flow can only be laminar, limiting the heat and mass transfer efficiency. If the optimal replenish rate is to be maintained across scales, the perfusion flow rate must increase linearly with the scale of the structure, leading to high shear stress and a considerable pressure drop. Typical strategies to solve such problems include enlarging the pore size of the scaffold material to improve the permeability, although this defeats the very purpose of the technology. There are some rudimentary studies on computational fluid dynamics (CFD) related to this topic (Hidalgo-Bastida, Thirunavukkarasu, Griffiths, Cartmell, & Naire, 2012), but for the most part, scaffolding used in tissue engineering is no more than a few millimeters in the direction of the media perfusion, and in the centimeter range perpendicular to the flow, with a perfusion velocity of around 1 cm/min or less (Gaspar, Gomide, & Monteiro, 2012). Recently, MacQueen et al. demonstrated bovine aortic smooth muscle cells and rabbit skeletal muscle myoblasts cultured on cross-linked gelatin fiber scaffolds, and those are of 1.5 mm thickness and 2 × 3 cm area. It is worth mentioning that the gelatin used in the aforementioned study was derived from animal products and it is edible, which is advantageous in comparison with other commonly used materials such as Poly-hydroxyl acids such as PLA and poly lactic-*co*-glycolic acid (PLGA) (MacQueen et al., 2019). Laminar flow with minimal back-mixing also means there is a steep gradient in dissolved oxygen (particularly if it is supplied from external oxygenation), CO_2 partial pressure, nutrients, and metabolic waste from the inlet to the outlet of the perfusion reactor, especially under high cell-density conditions. As such, homogeneity is almost certainly unachievable. Although some earlier patent applications envisioned commercial-scale cultured meat production based on 3D scaffolding (Vein, 2001, 2006), recent patent filings from more serious industrial players indicate that the focus is now shifting to alternative approaches (Gabor, Francoise, & Karoly, 2014). We tend to agree that due to the inherent limitations of mixing and mass transfer, etc., 3D scaffolding in its current form is unsuitable for large-scale implementation. Other techniques may emerge if there is sufficient demand for a highly structured product, but significant research and development is needed, and it may take another decade or longer before it is realized (Goodwin & Shoulders, 2013).

3.6. Suspension culture

The system of choice for large-scale ($> 1\ m^3$) mammalian cell cultures is the ubiquitous stirred tank reactor, where the cells are either freely suspended or attached to suspended microcarriers. The latter case applies to cultured meat, as myoblast cells are anchor-dependent (Verbruggen et al., 2018). Bubble columns (including airlift reactors) show some potential, owing to their perceived low shear operation in the absence of mechanical agitation (Merchuk, 1990). However, as it was later recognized that the local eddy energy dissipation associated with bursting bubbles is a major source of cell damage (Martens et al., 1996), interest in bubble columns for this particular application diminished. A more recent study found that an energy dissipation rate of 400 W/m^3 resulted in a 25% reduction in monoclonal antibody productivity with an industrial Chinese Hamster Ovary (CHO) cell line (Sieck et al., 2013), while an earlier numerical study revealed that the bursting of a 1 mm bubble results in a local energy dissipation rate of 10 MW/m^3, which can cause immediate cell death. Indeed, the "shear sensitivity" of mammalian cells is the root-cause that limits the scale and mass transfer efficiency, as shown in Fig. 2. Poor mass transfer necessitates the use of enriched air as the oxygen supply, which in turn results in high CO_2 partial pressure, insofar as the respiratory quotient (RQ) of animal cells is close to unity. Consequently, pure N_2 is used to strip the dissolved CO_2. While not a significant contributor to the cost of high added-value therapeutics and pharmaceuticals, this complexity can negatively affect the competiveness of cultured meat. In fact, Pluronic F68, a commonly used antifoam and shear protection agent, costs over CNY 42 per gram in China. When used at the recommended minimum concentration of 0.5 g/L (A.W. Nienow, 2006; Sieblist, Jenzsch, & Pohlscheidt, 2013), it adds CNY 21 per liter of reactor volume to the production cost. For comparison, 100 g of minced beef is sold for CNY 6–9.

At this juncture, it is natural to ask what a commercial-scale bioreactor for cultured meat production would look like. Based on the typical specific oxygen uptake of an animal cell, 10^{-16} mol/cell/s and a cell density of 10^8 cell/mL, a volumetric mass transfer coefficient of 250 1/h is required to maintain a dissolved oxygen level of 30–50% while achieving 50% oxygen conversion with no additional pressure applied to the reactor. The corresponding sterile air flow rate is about 1800 Nm^3/h, providing a superficial gas velocity of 0.012 m/s at the bottom and double that at the top of a 10 m tall reactor with a 5 m internal diameter (Fig. 3). The 1 atm hydrostatic pressure at the bottom of the reactor should not cause any damage to the cells (Takagi, Ohara, & Yoshida, 1995). It is worth noting is that with 50% oxygen conversion, the CO_2 concentration in the gas phase reaches 10.5%. If this is found to have a detrimental effect on the culture, CO_2 can be directly removed from the liquid phase using a selective CO_2 permeable membrane (e.g., Membrana Liqui-Cel® Membrane Contactors). Alternatively, the combination of a coarse sparger and higher aeration rate can provide the same oxygen transfer rate, at the expense of lower oxygen conversion, but with the additional benefit of lower CO_2 partial pressure. In addition to fast oxygen supply and CO_2 removal, the liquid medium needs to be replenished at a dilution rate of 1–2 1/day (Y. Zhang, Stobbe, Silvander, & Chotteau, 2015), whilst the cells have to be 100% retained. Therefore, an appropriate cell retention device, e.g., cross-flow filtration or continuous centrifugation, would be required to recycle the cells while the spent medium is discharged or treated and reused.

Although the k_La value in the above example is not particularly high, it still represents an increase of more than an order of magnitude over those typically seen in animal cell culture reactors. Since k_L is determined mostly by the physiochemical properties of the culture medium, the improvement in k_La would predominantly come from the specific mass transfer area, a, which in turn is a function of the gas holdup, ε_G (or volumetric gas fraction) and bubble size (d_b). Rational designs of such reactors using tools such as CFD are rather common nowadays (Villiger et al., 2018), and the only uncertainty is the shear sensitivity of the cells, especially with regard to bursting bubbles. There are reports on the tolerance of some commercial cell lines (Neunstoecklin et al., 2015; Neunstoecklin et al., 2016; Sieck et al., 2013), but no quantitative study has yet been performed on skeletal muscle cells.

3.7. Microcarriers

As briefly mentioned above, because myoblast cells are anchor-dependent, microcarriers must be used in conjunction with a stirred tank or bubble column bioreactor for suspended cultures. The use of microcarriers in animal cell cultivation has long been established (Van Wezel, 1967), and no major technical obstacles are expected to its application in cultured meat production. The liquid velocity and/or agitation speed required by the homogenization of the culture medium should exceed that required by microcarrier suspension at this scale, and this can be evaluated with CFD simulations (Delafosse, Loubière, Calvo, Toye, & Olmos, 2018). There is already some proof-of-concept experimental work that explores the potential use of microcarriers for cultured beef production, and what remains is a matter of screening for the optimal microcarrier material and structure, although no significant differences were observed among several commercially available products (Verbruggen et al., 2018), none of which was edible or biodegradable, though. Ideally, if the microcarrier is biodegradable and/or edible, it can be integrated into the final product, eliminating a downstream separation step. Materials suitable for this purpose include cross-linked pectin, such as pectin-thiopropionylamide (PTP), and RGD-containing polypeptide, such as thiolated cardosin A (Francoise S.M.,

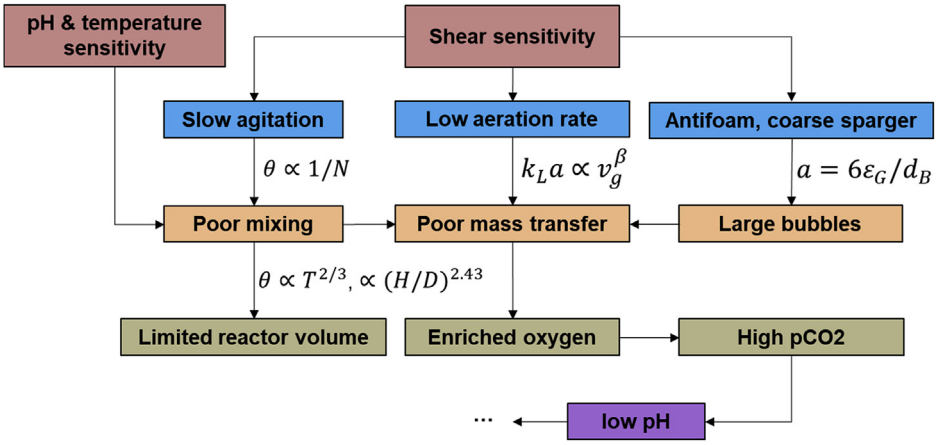

Fig. 2. Impact of perceived shear sensitivity of animal cells on reactor design and operation. θ: mixing time; N: agitation speed; T: tank diameter; H: liquid depth; $k_L a$: volumetric mass transfer coefficient; v_g: superficial gas velocity, i.e., volumetric gas flow rate divided by the reactor cross-sectional area; β: an empirical adjustable parameter; a: specific mass transfer area; ε_G: gas holdup, i.e., volumetric gas fraction; d_B: bubble diameter.

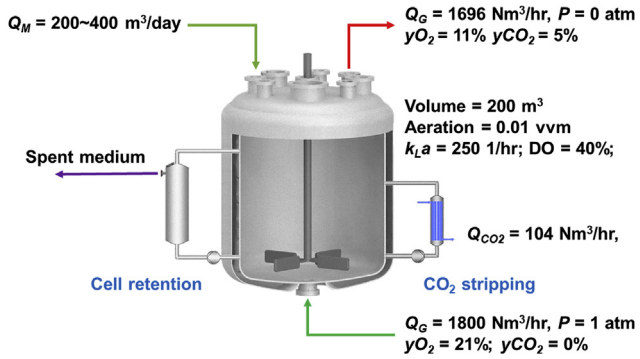

Fig. 3. Process flow diagram with key operating parameters for a potential large-scale cultured meat production reactor. Q_M: medium flow rate, Q_G: gas flow rate, P: pressure, $k_L a$: volumetric mass transfer coefficient, y_{O2}: O_2 volumetric fraction, y_{CO2}: CO_2 volumetric fraction, Q_{CO2}: CO_2 stripping rate.

Brendan P.P., Gabor F., & Andras F., 2017). Otherwise, the cells are to be harvested from the microcarriers, but we expect this to be much easier than that in the pharmaceutical industry, because, as food stock, the viability of the detached cells are of secondary importance. For instance, intensive agitation can be used, if necessary (A. W. Nienow, Rafiq, Coopman, & Hewitt, 2014). Interestingly, one conceptual flow diagram (Van der & Tramper, 2014) appears to suggest that muscle cells can be cultured in free suspension without the need for microcarriers, and, consequently, cells can be harvested by flocculation and sedimentation. We are unsure whether this was the result of the authors' negligence or whether they are basing their arguments on unpublished data.

3.8. Promising nutrient additives produced with synthetic biology

With the rapid development of cell factories and stem cell cultures, a small amount of cultured meat can be produced in a laboratory, however, it is at high cost and still a long way to commercialize it. Besides, the market acceptance of these products is also not enough (Bekker, Fischer, Tobi, & van Trijp, 2017; Verbeke et al., 2015). The main reason for this is that current artificial meat products do not realistically simulate the quality of real meat in a cost-effective and resource-efficient way. Therefore, it is necessary to satisfy preferences for muscle tissue with real color, nutrition, fragrance, and taste (Fig. 4).

On the one hand, the red color of real meat is given by the heme in hemoglobin or myoglobin (Sakata & Honokel, 2001; Salvador, Toldra, Pares, Carretero, & Saguer, 2009). However, artificial muscle tissues or vegetable proteins lack hemoglobin and myoglobin. Thus, in order to simulate the color of real meat, stable hemoglobin must be added to the products of artificial meat (Jin et al., 2018). Hemoglobin can be obtained from animal blood or plant tissue, but this method is time-consuming and labor-intensive (X. Zhang et al., 2017). Hence, much attention has been paid to biosynthesizing hemoglobin. To do so, microbial cells should first accumulate enough heme. In natural organisms, there are two pathways for heme biosynthesis (the C4 and C5 pathways) and the related enzymes involved in these two pathways and their coding genes have been clearly resolved (Layer, Reichelt, Jahn, & Heinz, 2010). Based on this information, a small amount of heme can be synthesized using the C4 pathway in *Escherichia coli* (Pranawidjaja, Choi, Lay, & Kim, 2015). However, this strategy requires the addition of glycine and succinic acid as substrates, and this is unsuitable for large-scale fermentation. In the latest research, without the addition of substrates, extracellular heme production was achieved in *E. coli* using the C5 pathway through the inhibition of heme degradation and by-product formation (X. R. Zhao, Choi, & Lee, 2018).

On the basis of adequate heme in the microbial cells, hemoglobin can be further synthesized from different sources. Currently, most research focuses on synthesizing human hemoglobin because it can be utilized as hemoglobin-based oxygen carriers (Njoku, St Peter, & Mackenzie, 2015). Although human hemoglobin has already been synthesized both in *E. coli* (Natarajan et al., 2011) and *Saccharomyces cerevisiae* (Liu, Martinez, Liu, Petranovic, & Nielsen, 2014; Martinez, Liu, Petranovic, & Nielsen, 2015), food-grade *S. cerevisiae* is more suitable for the synthesis of eukaryotic hemoglobins. The proportion of heterologous human hemoglobin in intracellular protein can reach 7% in *S. cerevisiae* by enhancing the heme synthesis pathway (Liu et al., 2014) and regulating the oxygen detection system (Martinez et al., 2015). Besides human hemoglobin, the American company Impossible Foods Inc. successfully synthesized soybean hemoglobin in *Pichia pastoris*. Nonetheless, since *P. pastoris* is not a food-grade host and the purity of soybean hemoglobin used in the production of beef burgers can reach no more than 65%, there are certain food security risks with producing artificial meat (Jin et al., 2018). In addition, soybean hemoglobin is notably different from animal hemoglobin in structure and function. Therefore, it is necessary to use food-grade strains to produce hemoglobin from different animal sources (swine, bovine, sheep, etc.) using metabolic engineering strategies.

On the other hand, the scent of meat is pleasurable and promotes the absorption of nutrients. In order to obtain higher market acceptance, artificial meat must have a real and attractive scent. By comparing the chemical composition of raw and cooked meat, it was found that the main aromatic substances in meat are several sulfur-containing

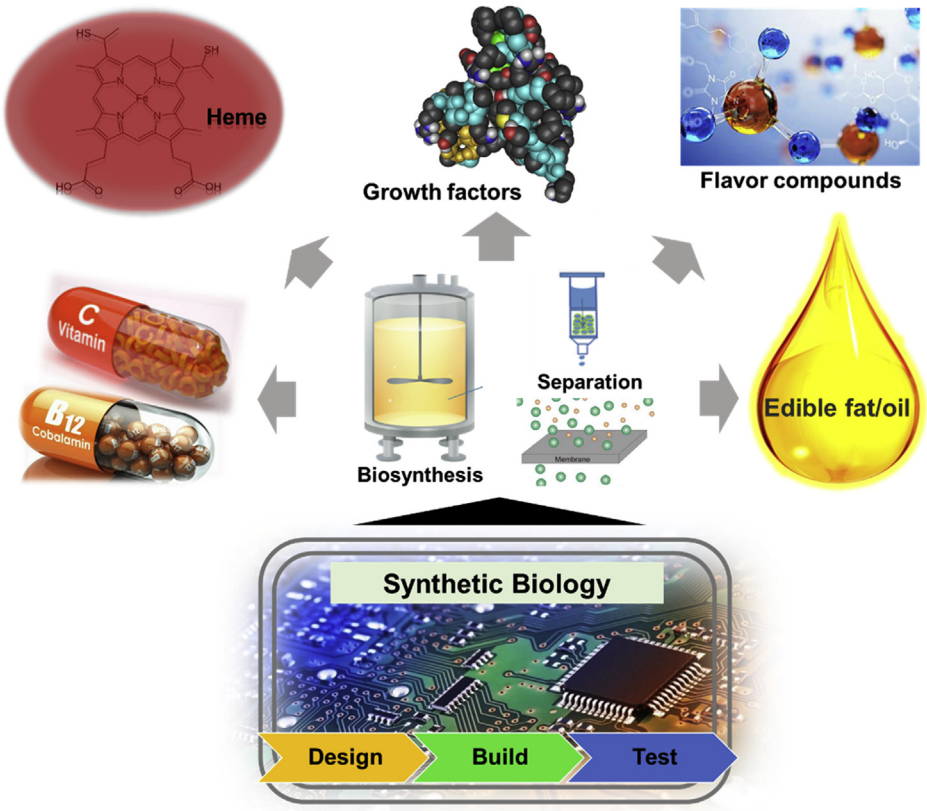

Fig. 4. Synthetic biology-based bioengineering applied to cultured meat production and optimization. Synthetic biology designed microbes have been used to produce individual molecules and can be easily scaled up for commercial production, such as food additives, enzymes and other compounds.

and nitrogen-containing compounds formed from amino acids and sugars at high temperatures, with traces of aldehydes, ketones, alcohols, and furans (Kang, Alim, & Song, 2019; Yu et al., 2016). Thus, in recent years, various aromatic substances have been produced by reacting enzymatic hydrolysate in animal or vegetable proteins with amino acids and reducing saccharides (Lotfy, Fadel, El-Ghorab, & Shaheen, 2015; Wang & Cha, 2018).

Beef-like aromatic substances, including thiol, pyrazines, thiazoles, and disulphides, can be prepared with conventional or microwave heating of the enzymatic hydrolysate of mushroom protein with other precursors (Lotfy et al., 2015). Moreover, as many as 57 volatile flavor compounds contributing to meaty odor can be formed by mixing the enzymatic hydrolysates of soy sauce residue with defatted soybean (Wang & Cha, 2018). In addition, response surface methodology analysis was used to optimize the conditions of the Maillard reaction to form aromatic substances from many different sources (Yang, Song, Chen, & Zou, 2011).

Besides aromatic substances, an appropriate amount of fatty acid is also needed to endow artificial meat with the unique flavor of meat. Applying efficient, integrated expression cassettes containing two desaturase genes from *Mortierella alpina*, ample amounts of linolenic acid were synthesized from intracellular precursors in *Yarrowia lipolytica* (linoleic acid and oleic acid) (Chuang et al., 2010). Further, eicosadienoic acid, eicosatrienoic acid, and eicosapentaenoic acid can be synthesized from inexpensive raw materials in *Y. lipolytica* after optimizing the culture conditions (Poli et al., 2014). At present, the yield of unsaturated fatty acids in commercial ester-producing strains can reach more than 50% of the total intracellular fatty acids (Lazar, Liu, & Stephanopoulos, 2018). In addition, linolenic acid and their derived esters can be efficiently extracted from cells (Yook, Kim, Woo, Um, & Lee, 2019) and used as food additives to improve the taste of artificial meat.

3.9. Customized production of artificial meat with 3D printing

In recent years, with improvements in public living standards, there is growing demand for meat products that are customized to the different dietary habits. As there are significant differences in taste and flavor in the meat of different parts of animals, the proportion of main components in the desired products should first be determined. The physical composition and nutrient content of different cuts of beef can be obtained by applying near-infrared spectroscopy and mass spectrometry (Schonfeldt, Naude, & Boshoff, 2010). Furthermore, upgraded equipment and artificial intelligence can be used for rapid and accurate analysis of the components of different meats, laying the foundation for the production of customized fast-consumption artificial meat (Perez et al., 2018). Based on these results, the proportion of main components was optimized with a multi-dimensional artificial neural network to construct a qualitative model for special artificial meat (Zou et al., 2018).

In addition, the shape of artificial meat is an important and influential factor for consumer acceptance. At present, the products of artificial meat are too loose to generate the feeling of real chewing. Therefore, three-dimensional printing is urgently needed to reshape the structure of artificial meat, for a vivid replication of the compact and elastic structure of real meat (Godoi, Prakash, & Bhandari, 2016). Using modeling software (Rimann, Bono, Annaheim, Bleisch, & Graf-Hausner, 2016) and spraying equipment (Gunther, Heymel, Gunther, & Ederer, 2014), the three-dimensional structure of artificial meat can be designed and the raw materials and auxiliary materials can be thoroughly mixed and organized. Moreover, the latest three-dimensional printing technology enables the fabrication of flexible artificial vessels (Attalla, Puersten, Jain, & Selvaganapathy, 2018) and local control of the graininess and toughness of artificial meat (Lueders, Jastram, Hetzer, & Schwandt, 2014; Saratti, Rocca, & Krejci, 2019), to better simulate the

three-dimensional structure of real meat.

4. Prospect and conclusions

The growing global demand for meat is a considerable challenge, owing to increasingly serious resource and environmental constraints. Although cultured meat is considered a promising alternative to conventional meat, it is still in its early stages and lacks a solid foundation: artificial meat lacks the necessary nutrients, it is costly, and food safety certification has not yet embraced it. Moreover, there are fundamental issues that need to be resolved, pertaining to social and ethical constraints, efficient tissue engineering, fine-tuned culture conditions, large-scale bioreactors, and the development of cost-effective and safe serum-free culture media (Tuomisto, 2019).

Another important issue concerns the acceptance of cultured meat by the public. Some reports show that even though people understand the need to develop sustainable meat alternatives, they remain pessimistic regarding the challenges of scaled-up production, cost, and food safety, which still need to develop lots of security research and promotion for healthy concept of cultured meat (Tiberius et al., 2019). Based on existing research, there are some main risk factors for cultured meat, such as food safety certification of components used in cultured meat, genetic engineering applied in cultured meat.

In general, however, with increasing demand and further development of biotechnologies, cultured meat may ultimately compete with conventional meat as a slaughter-free and sustainable choice, with the potential to relieve the stress from an increasing population and demand for meat (Bhat et al., 2015).

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2018YFC1604102, 2017YFC1600403), the National Science Fund for Excellent Young Scholars (21822806), the Fundamental Research Funds for the Central Universities (JUSRP51701A), the National First-class Discipline Program of Light Industry Technology and Engineering (LITE2018-08), the Distinguished Professor Project of Jiangsu Province, and the 111 Project (111-2-06).

References

Alexander, R. (2011). *In vitro meat: A vehicle for the ethical rescaling of the factory farming industry and in vivo testing or an intractable enterprise? Intersect, Vol. 4*, 42–47.

Amit, M., Carpenter, M. K., Inokuma, M. S., Chiu, C. P., Harris, C. P., Waknitz, M. A., et al. (2000). Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Developmental Biology, 227*, 271–278.

Arshad, M. S., Javed, M., Sohaib, M., Saeed, F., Imran, A., & Amjad, Z. (2017). Tissue engineering approaches to develop cultured meat from cells: A mini review. *Cogent Food And Agriculture, 3*, 1–11.

Aswad, H., Jalabert, A., & Rome, S. (2016). Depleting extracellular vesicles from fetal bovine serum alters proliferation and differentiation of skeletal muscle cells in vitro. *BMC Biotechnology, 16*, 32.

Attalla, R., Puersten, E., Jain, N., & Selvaganapathy, P. R. (2018). 3D bioprinting of heterogeneous bi- and tri-layered hollow channels within gel scaffolds using scalable multi-axial microfluidic extrusion nozzle. *Biofabrication, 11*, 015012.

Bekker, G. A., Fischer, A. R. H., Tobi, H., & van Trijp, H. C. M. (2017). Explicit and implicit attitude toward an emerging food technology: The case of cultured meat. *Appetite, 108*, 245–254.

Bellarby, J., Tirado, R., Leip, A., Weiss, F., Lesschen, J. P., & Smith, P. (2013). Livestock greenhouse gas emissions and mitigation potential in Europe. *Global Change Biology, 19*, 3–18.

Benjaminson, M. A., Gilchriest, J. A., & Lorenz, M. (2002). In vitro edible muscle protein production system (MPPS): Stage 1, fish. *Acta Astronautica, 51*, 879–889.

Bhat, Z. F., & Bhat, H. (2011a). Animal-free meat biofabrication. *American Journal of Food Technology, 6*, 441–459.

Bhat, Z. F., & Bhat, H. (2011b). Tissue engineered meat-Future meat. *Journal of Stored Products and Postharvest Research, 2*, 1–10.

Bhat, Z. F., & Fayaz, H. (2011). Prospectus of cultured meat—advancing meat alternatives. *Journal of Food Science & Technology, 48*, 125–140.

Bhat, Z. F., Kumar, S., & Bhat, H. F. (2017). In vitro meat: A future animal-free harvest. *Critical Reviews in Food Science and Nutrition, 57*, 782–789.

Bhat, Z. F., Kumar, S., & Fayaz, H. (2015). In vitro meat production: Challenges and benefits over conventional meat production. *Journal of Integrative Agriculture, 14*, 241–248.

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

Bogliotti, Y. S., Wu, J., Vilarino, M., Okamura, D., Soto, D. A., Zhong, C., et al. (2018). Efficient derivation of stable primed pluripotent embryonic stem cells from bovine blastocysts. *Proceedings of the National Academy of Sciences of the U S A, 115*, 2090–2095.

Brunner, D., Frank, J., Appl, H., Schöffl, H., Pfaller, W., & Gstraunthaler, G. (2010). Serum-free cell culture: The serum-free media interactive online database. *ALTEX, 27*, 53–62.

Chuang, L. T., Chen, D. C., Nicaud, J. M., Madzak, C., Chen, Y. H., & Huang, Y. S. (2010). Co-expression of heterologous desaturase genes in *Yarrowia lipolytica*. *New Biotech, 27*, 277–282.

Cravero, D., Martignani, E., Miretti, S., Accornero, P., Pauciullo, A., Sharma, R., et al. (2015). Generation of induced pluripotent stem cells from bovine epithelial cells and partial redirection toward a mammary phenotype in vitro. *Cellular Reprogramming, 17*, 211–220.

Datar, I., & Betti, M. (2010). Possibilities for an in vitro meat production system. *Innovative Food Science & Emerging Technologies, 11*, 13–22.

Delafosse, A., Loubière, C., Calvo, S., Toye, D., & Olmos, E. (2018). Solid-liquid suspension of microcarriers in stirred tank bioreactor-Experimental and numerical analysis. *Chemical Engineering Science, 180*, 52–63.

Ebeling, A. H. (1914). The effect of the variation in the osmotic tension and of the dilution of culture media on the cell proliferation of connective tissue. *Journal of Experimental Medicine, 20*, 130–139.

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

Fao (2006). *Livestock's long shadow-Environmental issues and options.* FAO publications.

Flickinger, M. C., Hu, W., Zhou, T.-C., Zhou, W.-W., & Zhong, J.-J. (2010). *Bioreactors, cell culture, commercial production.* Hoboken, NJ, USA: John Wiley & Sons, Inc.

Francoise S.M., Brendan P.P., Gabor F., & Andras F. (2017). Edible and animal-product-free microcarriers for engineered meat. In (Vol. 9 , 752 , 12). US Patent.

Gabor, F., Francoise, M., & Karoly, R. J. (2014). Engineered comestible meat. In U. S. Patent (Ed.). *The curators of the university of Missouri*.

Gaspar, D. A., Gomide, V., & Monteiro, F. J. (2012). The role of perfusion bioreactors in bone tissue engineering. *Biomatter, 2*, 167–175.

Geraghty, R. J., Capes-Davis, A., Davis, J. M., Downward, J., Freshney, R. I., Knezevic, I., et al. (2014). Guidelines for the use of cell lines in biomedical research. *British Journal of Cancer, 111*, 1021–1046.

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., et al. (2013). *Tackling climate change through livestock: A global assessment of emissions and mitigation opportunities.* Rome, Italy: Food and Agriculture Organization of the United Nations (FAO).

Godoi, F. C., Prakash, S., & Bhandari, B. R. (2016). 3D printing technologies applied for food design: Status and prospects. *Journal of Food Engineering, 179*, 44–54.

Goodwin, J. N., & Shoulders, C. W. (2013). The future of meat: A qualitative analysis of cultured meat media coverage. *Meat Science, 95*, 445–450.

Gunther, D., Heymel, B., Gunther, J. F., & Ederer, I. (2014). Continuous 3D-printing for additive manufacturing. *Rapid Prototyping Journal, 20*, 320–327.

Hidalgo-Bastida, L. A., Thirunavukkarasu, S., Griffiths, S., Cartmell, S. H., & Naire, S. (2012). Modeling and design of optimal flow perfusion bioreactors for tissue engineering applications. *Biotechnology and Bioengineering, 109*, 1095–1099.

Hocquette, J. F., Gondret, F., Baéza, E., Médale, F., Jurie, C., & Pethick, D. W. (2010). Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal, 4*, 303–319.

Jin, Y., He, X. Y., Andoh-Kumi, K., Fraser, R. Z., Lu, M., & Goodman, R. E. (2018). Evaluating potential risks of food allergy and toxicity of soy leghemoglobin expressed in *Pichia pastoris*. *Molecular Nutrition & Food Research, 62* 1700297.

Kang, L., Alim, A., & Song, H. (2019). Identification and characterization of flavor precursor peptide from beef enzymatic hydrolysate by Maillard reaction. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences, 1104*, 176–181.

Kruk, Z. A., Bottema, M. J., Reyes-Veliz, L., Forder, R. E. A., Pitchford, W. S., & Bottema, C. D. K. (2018). Vitamin A and marbling attributes: Intramuscular fat hyperplasia effects in cattle. *Meat Science, 137*, 139–146.

Layer, G., Reichelt, J., Jahn, D., & Heinz, D. W. (2010). Structure and function of enzymes in heme biosynthesis. *Protein Science, 19*, 1137–1161.

Lazar, Z., Liu, N., & Stephanopoulos, G. (2018). Holistic approaches in lipid production by *Yarrowia lipolytica*. *Trends in Biotechnology, 36*, 1157–1170.

Leong, D. S., Tan, J. G., Chin, C. L., Mak, S. Y., Ho, Y. S., & Ng, S. K. (2017). Evaluation and use of disaccharides as energy source in protein-free mammalian cell cultures. *Scientific Reports, 7* 45216.

Liu, L. F., Martinez, J. L., Liu, Z. H., Petranovic, D., & Nielsen, J. (2014). Balanced globin protein expression and heme biosynthesis improve production of human hemoglobin in *Saccharomyces cerevisiae*. *Metabolic Engineering, 21*, 9–16.

Lotfy, S. N., Fadel, H. H., El-Ghorab, A. H., & Shaheen, M. S. (2015). Stability of encapsulated beef-like flavourings prepared from enzymatically hydrolysed mushroom proteins with other precursors under conventional and microwave heating. *Food Chemistry, 187*, 7–13.

Lueders, C., Jastram, B., Hetzer, R., & Schwandt, H. (2014). Rapid manufacturing techniques for the tissue engineering of human heart valves. *European Journal of Cardio-Thoracic Surgery, 46*, 593–601.

MacQueen, L. A., Alver, C. G., Chantre, C. O., Ahn, S., Cera, L., Gonzalez, G. M., et al. (2019). Muscle tissue engineering in fibrous gelatin: Implications for meat analogs. *NPJ Sci Food, 3*.

Martens, D. E., Nollen, E. A. A., Hardeveld, M., van der Velden-de Groot, C. A. M., de Gooijer, C. D., Beuvery, E. C., et al. (1996). Death rate in a small air-lift loop reactor of vero cells grown on solid microcarriers and in macroporous microcarriers. *Cytotechnology, 21*, 45–59.
Martinez, J. L., Liu, L. F., Petranovic, D., & Nielsen, J. (2015). Engineering the oxygen sensing regulation results in an enhanced recombinant human hemoglobin production by *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering, 112*, 181–188.
Masters, J. R., & Stacey, G. N. (2007). Changing medium and passaging cell lines. *Nature Protocols, 2*, 2276–2284.
Mattick, C. S., Landis, A. E., Allenby, B. R., & Genovese, N. J. (2015). Anticipatory life cycle analysis of in vitro biomass cultivation for cultured meat production in the United States. *Environmental Science & Technology, 49*, 11941–11949.
Mauro, A. (1961). Satellite cell of skeletal muscle fibers. *The Journal of Biophysical and Biochemical Cytology, 9*, 493–495.
Merchuk, J. (1990). Why use air-lift bioreactors? *Trends in Biotechnology, 8*, 66–71.
Miki, H., & Takagi, M. (2015). Design of serum-free medium for suspension culture of CHO cells on the basis of general commercial media. *Cytotechnology, 67*, 689–697.
Natarajan, C., Jiang, X. B., Fago, A., Weber, R. E., Moriyama, H., & Storz, J. F. (2011). Expression and purification of recombinant hemoglobin in *Escherichia coli*. *PloS One, 6*, e20176.
Neunstoecklin, B., Stettler, M., Solacroup, T., Broly, H., Morbidelli, M., & Soos, M. (2015). Determination of the maximum operating range of hydrodynamic stress in mammalian cell culture. *Journal of Biotechnology, 194*, 100–109.
Neunstoecklin, B., Villiger, T. K., Lucas, E., Stettler, M., Broly, H., Morbidelli, M., et al. (2016). Pilot-scale verification of maximum tolerable hydrodynamic stress for mammalian cell culture. *Applied Microbiology and Biotechnology, 100*, 3489–3498.
Nienow, A. W. (2006). Reactor engineering in large scale Animal cell culture. *Cytotechnology, 50*, 9.
Nienow, A. W., Rafiq, Q. A., Coopman, K., & Hewitt, C. J. (2014). A potentially scalable method for the harvesting of hMSCs from microcarriers. *Biochemical Engineering Journal, 85*, 79–88.
Njoku, M., St Peter, D., & Mackenzie, C. F. (2015). Haemoglobin-based oxygen carriers: Indications and future applications. *British Journal of Hospital Medicine, 76*, 78–83.
O'Riordan, K., Fotopoulou, A., & Stephens, N. (2017). The first bite: Imaginaries, promotional publics and the laboratory grown burger. *Public Understanding of Science, 26*, 148–163.
Oosterhuis, N. (2018). Bioreactor design for cultured meat, where to go? *Paper presented at the 4th international conference on cultured meat* Maastricht, Netherlands.
Park, Y. H., Gong, S. P., Kim, H. Y., Kim, G. A., Choi, J. H., Ahn, J. Y., et al. (2013). Development of a serum-free defined system employing growth factors for preantral follicle culture. *Molecular Reproduction and Development, 80*, 725–733.
Perez, I. M. N., Badaro, A. T., Barbon, S., Barbon, A. P. A. C., Pollonio, M. A. R., & Barbin, D. F. (2018). Classification of chicken parts using a portable near-infrared (NIR) spectrophotometer and machine learning. *Applied Spectroscopy, 72*, 1774–1780.
Poli, J. S., da Silva, M. A. N., Siqueira, E. P., Pasa, V. M. D., Rosa, C. A., & Valente, P. (2014). Microbial lipid produced by *Yarrowia lipolytica* QU21 using industrial waste: A potential feedstock for biodiesel production. *Bioresource Technology, 161*, 320–326.
Post, M. (2012). Cultured meat from stem cells: Challenges and prospects. *Meat Science, 92*, 297–301.
Post, M. (2014). Cultured beef: Medical technology to produce food. *Journal of the Science of Food and Agriculture, 94*, 1039–1041.
Pranawidjaja, S., Choi, S. I., Lay, B. W., & Kim, P. (2015). Analysis of heme biosynthetic pathways in a recombinant *Escherichia coli*. *Journal of Microbiology and Biotechnology, 25*, 880–886.
Ramboer, E., De Craene, B., De Kock, J., Vanhaecke, T., Berx, G., Rogiers, V., et al. (2014). Strategies for immortalization of primary hepatocytes. *Journal of Hepatology, 61*, 925–943.
Rimann, M., Bono, E., Annaheim, H., Bleisch, M., & Graf-Hausner, U. (2016). Standardized 3D bioprinting of soft tissue models with human primary cells. *Journal of Laboratory Automation, 21*, 496–509.
Sakata, R., & Honokel, K. O. (2001). Investigation the physical and chemical properties of the red pigment of meat products. *Fleischwirtschaft, 81*, 182–190.
Salvador, P., Toldra, M., Pares, D., Carretero, C., & Saguer, E. (2009). Color stabilization of porcine hemoglobin during spray-drying and powder storage by combining chelating and reducing agents. *Meat Science, 83*, 328–333.
Saratti, C. M., Rocca, G. T., & Krejci, I. (2019). The potential of three-dimensional printing technologies to unlock the development of new 'bio-inspired' dental materials: An overview and research roadmap. *Journal of Prosthodontic Research, 63*, 131–139.
Schonfeldt, H. C., Naude, R. T., & Boshoff, E. (2010). Effect of age and cut on the nutritional content of South African beef. *Meat Science, 86*, 674–683.
Seman, D. L., Quickert, S. C., Borger, A. C., & Meyer, J. D. (2008). Inhibition of Listeria monocytogenes growth in cured ready-to-eat meat products by use of sodium benzoate and sodium diacetate. *Journal of Food Protection, 71*, 1386–1392.
Shay, J. W., & Wright, W. E. (2000). Hayflick, his limit, and cellular ageing. *Nature Reviews Molecular Cell Biology, 1*, 72–76.
Sieblist, C., Jenzsch, M., & Pohlscheidt, M. (2013). Influence of pluronic F68 on oxygen mass transfer. *Biotechnology Progress, 29*, 1278–1288.
Sieck, J. B., Cordes, T., Budach, W. E., Rhiel, M. H., Suemeghy, Z., Leist, C., et al. (2013). Development of a Scale-Down Model of hydrodynamic stress to study the performance of an industrial CHO cell line under simulated production scale bioreactor conditions. *Journal of Biotechnology, 164*, 41–49.
Specht, E. A., Welch, D. R., Rees Clayton, E. M., & Lagally, C. D. (2018). Opportunities for applying biomedical production and manufacturing methods to the development of the clean meat industry. *Biochemical Engineering Journal, 132*, 161–168.
Stephens, N., Di Silvio, L., Dunsford, I., Ellis, M., Glencross, A., & Sexton, A. (2018). Bringing cultured meat to market: Technical, socio-political, and regulatory challenges in cellular agriculture. *Trends in Food Science & Technology, 78*, 155–166.
Stern-Straeter, J., Bonaterra, G. A., Juritz, S., Birk, R., Goessler, U. R., Bieback, K., et al. (2014). Evaluation of the effects of different culture media on the myogenic differentiation potential of adipose tissue- or bone marrow-derived human mesenchymal stem cells. *International Journal of Molecular Medicine, 33*, 160–170.
Takagi, M., Ohara, K., & Yoshida, T. (1995). Effect of hydrostatic pressure on hybridoma cell metabolism. *Journal of Fermentation and Bioengineering, 80*, 619–621.
Takahashi, M., Makino, S., Kikkawa, T., & Osumi, N. (2014). Preparation of rat serum suitable for mammalian whole embryo culture. *Journal of Visualized Experiments, 90*, e51969.
Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*, 663–676.
Tan, K. Y., Teo, K. L., Lim, J. F., Chen, A. K., Choolani, M., Reuveny, S., et al. (2015). Serum-free media formulations are cell line-specific and require optimization for microcarrier culture. *Cytotherapy, 17*, 1152–1165.
Tiberius, V., Borning, J., & Seeler, S. (2019). Setting the table for meat consumers: An international delphi study on in vitro meat. *NPJ Sci Food, 3*.
Tuomisto, H. L. (2019). The eco-friendly burger: Could cultured meat improve the environmental sustainability of meat products? *EMBO Reports, 20*, e47395.
Tuomisto, H. L., & Teixeira de Mattos, M. J. (2011). Environmental impacts of cultured meat production. *Environmental Science & Technology, 45*, 6117–6123.
Van Wezel, A. L. (1967). Growth of cell-strains and primary cells on micro-carriers in homogeneous culture. *Nature, 216*, 64–65.
Van der, W. C., & Tramper, J. (2014). Cultured meat: Every village its own factory? *Trends in Biotechnology, 32*, 294–296.
Vein, J. (2001). *Method for producing tissue engineered meat for consumption.* United States Patent instead of (U.S.P) (USA).
Vein, J. (2006). *Tissue engineered meat for consumption and a method for producing tissue engineered meat for consumption.* W. I. P. Organization.
Verbeke, W., Marcu, A., Rutsaert, P., Gaspar, R., Seibt, B., Fletcher, D., et al. (2015). Would you eat cultured meat?': Consumers' reactions and attitude formation in Belgium, Portugal and the United Kingdom. *Meat Science, 102*, 49–58.
Verbruggen, S., Luining, D., van Essen, A., & Post, M. J. (2018). Bovine myoblast cell production in a microcarriers-based system. *Cytotechnology, 70*, 503–512.
Villiger, T. K., Neunstoecklin, B., Karst, D. J., Lucas, E., Stettler, M., Broly, H., et al. (2018). Experimental and CFD physical characterization of animal cell bioreactors: From micro- to production scale. *Biochemical Engineering Journal, 131*, 84–94.
Wang, W., & Cha, Y. J. (2018). Volatile compounds in seasoning sauce produced from soy sauce residue by reaction flavor technology. *Preventive Nutrition and Food Science, 23*, 356–363.
Warner, R. D. (2019). Review: Analysis of the process and drivers for cellular meat production. *Animal, 13*, 3041–3058.
Webster, R. (2002). The importance of animal influenza for human disease. *Vaccine, 20*, S16–S20.
Wolfson, W. (2002). Raising the steaks. *New Sci, 176*, 60–63.
Wu, S. M., & Hochedlinger, K. (2011). Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nature Cell Biology, 13*, 497–505.
Yamazoe, H., Ichikawa, T., Hagihara, Y., & Iwasaki, Y. (2016). Generation of a patterned co-culture system composed of adherent cells and immobilized nonadherent cells. *Acta Biomaterialia, 31*, 231–240.
Yang, C., Song, H. L., Chen, F., & Zou, T. T. (2011). Response surface methodology for meat-like odorants from the maillard reaction with glutathione II: The tendencies analysis of meat-like donors. *Journal of Food Science, 76*, C1267–C1277.
Yook, S. D., Kim, J., Woo, H. M., Um, Y., & Lee, S. M. (2019). Efficient lipid extraction from the oleaginous yeast *Yarrowia lipolytica* using switchable solvents. *Renewable Energy, 132*, 61–67.
Yu, L., Gao, C., Zeng, M., He, Z., Wang, L., Zhang, S., et al. (2016). Effects of raw meat and process procedure on N-epsilon-carboxymethyllysine and N-epsilon-carboxyethyllysine formation in meat products. *Food Science and Biotechnology, 25*, 1163–1168.
Zhang, Y., Stobbe, P., Silvander, C., & Chotteau, V. (2015). Very high cell density perfusion of CHO cells anchored in a non-woven matrix-based bioreactor. *Journal of Biotechnology, 213*, 28–41.
Zhang, X., Tan, J., Xu, X., Shi, F., Li, G., & Yang, Y. (2017). A coordination polymer based magnetic adsorbent material for hemoglobin isolation from human whole blood, highly selective and recoverable. *Journal of Solid State Chemistry, 253*, 219–226.
Zhao, X. R., Choi, K., & Lee, S. Y. (2018). Metabolic engineering of *Escherichia coli* for secretory production of free haem. *Nature Catalysis, 1*, 720–728.
Zhao, L., Wang, Z., Zhang, J., Yang, J., Gao, X., Wu, B., et al. (2017). Characterization of the single-cell derived bovine induced pluripotent stem cells. *Tissue and Cell, 49*, 521–527.
Zou, Y., Zhang, K., Zhang, X., Li, P., Zhang, M., Liu, F., et al. (2018). Optimization of goose breast meat tenderness by rapid ultrasound treatment using response surface methodology and artificial neural network. *Animal Science Journal, 89*, 1339–1347.