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REVIEW ARTICLE

Cultivated meat: recent technological developments, current market and future challenges

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Highlights

- Cultivated Meat is a potential candidate to (partially) substitute animal meat in the future decades;
- Current technological developments and tendencies are presented;
- Bioeconomy and sustainable aspects of CM production are discussed;
- Cultivated Meat Market is growing fast in the last few years;
- Challenges regarding CM production comprises public acceptance and social, economical and environmental aspects.

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KEYWORDS
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Recent patents.

Abstract: The increasing demand for food, the debates regarding the ethics involved in slaughtering animals and the many associated environmental issues promote the emergence of an interesting question: is it possible to substitute conventional meat? In this context, Cultivated Meat (CM) is a promising alternative to replace meat, or at least to complement protein nutrition for humans. This overview aims to show the current technological developments for the production of CM, starting with the tissue engineering used to collect, grow and differentiate the cells, and also the characteristics of matrixes, culture media, types of bioreactors and techniques employed for cell cultivation. In addition, bioeconomy and sustainability issues are discussed, as well as social aspects and policy regulation. Furthermore, the fast growing market is presented, starting with the first meat ball in 2016, passing through some examples of recent funding and operating companies and start-ups, the continuous efforts to lower production costs, besides the most recent patented processes. Finally, and in the light of recent developments, future challenges and expectations for the future of CM are discussed, such as tissue engineering bottlenecks, bioreactor design optimization and public acceptance issues.

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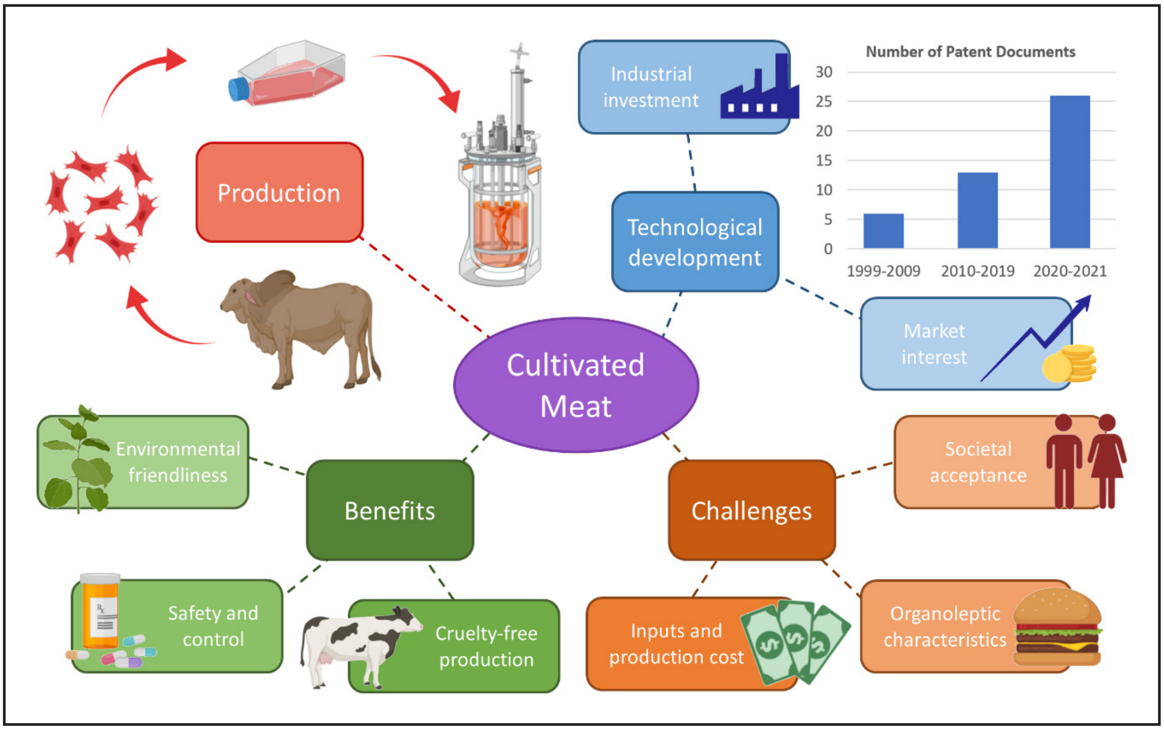
GRAPHICAL ABSTRACT



Introduction

The continuous growth of world population stimulates the debate of the necessity to enhance global food production and find alternative sources of food. Meat is an important protein source, and it is considered one of the most important food resources in many cultures. In the year of 2014, the consumption of meat reached 300 million tons and the growth projection for consumption is 76% by 2050 (Guan et al., 2021). Tilman et al. (2011) also forecasted a great increase in meat consumption, around 100-110% between 2005 and 2050.

On the other side, the consumption of meat implies the slaughtering of animals, which is not considered acceptable by many people, especially in some regions or cultures. Also, meat consumption is responsible for considerable environmental pollution and for the use of great amounts of natural resources. In this context, there is an emerging interest in alternatives or complements for conventional meat, and one of the most important ones, elected as “Top 10 rising technologies of 2018” by the world Economic Forum, is the so called “animal cell meat” (Guan et al., 2021; Ng & Kurisawa, 2021; Zhang et al., 2020).

The cultivated meat (CM), also called “animal cell meat”, or “artificial meat” or “synthetic meat”, or “in vitro meat” or “clean meat” or lab meat” or “cell based meat”, among others, can be defined in short words as meat obtained from the *ex-situ* culture of animal cells. These cells are usually obtained by biopsy of animals, then cultivated in proper conditions and with proper nutrients and energetic sources, giving place to complex structures similar to muscle tissues (fat cells, muscle cells and other components found in animal meat). Further processing usually has the objective to obtain a final product with similar characteristics to the conventional meat and comprises molding, seasoning and coloring (Jairath et al., 2021; Warner, 2019; Zhang et al., 2020).

Despite its incipient stage, CM is attracting much attention from scientists, investors and entrepreneurs. The costs for its production are still considerably high and much technological improvement is still necessary, but much effort have been made in recent years to bring to reality its industrialization and commercialization within a few years from now. Just between 2016 and 2020, around 460 million dollars have been invested in CM companies (Guan et al., 2021).

Considering the importance of CM, the main aims of this article are to present an overview of current technologies and techniques employed for its obtainment; to analyze its advantages and disadvantages considering bioeconomy, sustainability and also human and social aspects; to briefly present the current market and patented processes; and also to discuss the challenges and the future of this rising thematic.

Cell expansion and differentiation for cultivated meat production

The upstream process of CM may be categorized by obtaining cells, proliferating and differentiating them *ex situ*, and processing to the final meat product (Guan et al., 2021). It is notable that some key technologies applied for

CM production process derive from mammalian cell culture and tissue engineering developments achieved in the biopharmaceutical industry.

Muscle tissue from animals comprise several different cell types. Regarding the relevance to obtain CM, the main cells are skeletal muscle myocytes, adipocytes (fat cells), and fibroblasts (connective tissue) (Warner, 2019). Strategies to obtain these cell cultures *in vitro* are mainly based on using primary cell lineages extracted from animals, *ex situ*. Such samples include differentiated cells and stem cells, both primary cells exhibiting limited proliferation *ex vivo*, as opposed to immortalized cell lineages (Ng & Kurisawa, 2021). Embryonic stem cells are considered pluripotent stem cells and a good option for the CM process; however, although possible, it is challenging to obtain them and to efficiently differentiate them into the desired cell lines (Bogliotti et al., 2018). Otherwise, a more viable alternative are the adult stem cells, which are found in a variety of tissues, but exhibit limited differentiation ability.

Myoblasts, myosatellites, myocytes (myotubes and myofibers), adipocytes, adipose-derived stem cells, fibroblasts and iPSCs (induced pluripotent stem cells) are examples of cell types more extensively being considered for the CM process (Allan et al., 2019; O'Neill et al., 2021). Myosatellite cells are adult stem cells acting on muscle regeneration. These cell can differentiate into myocytes *ex vivo*. Also, adipose tissue-derived adult stem cells can differentiate into adipocytes *ex vivo* (Arshad et al., 2017).

Different cell lineages exhibit specific properties. In order to establish and optimize the CM production, the relevant features must be considered, such as: proliferation and differentiation methods, culture medium requirements, dry and wet mass, protein and water content, specific growth rate, and anchorage dependence (Allan et al., 2019). Culture media free of animal ingredients are essential, specially media free of animal serum, as it can represent the major production cost (Ng & Kurisawa, 2021). In fact, the proposal of a transition to CM seems only coherent if no animal input is needed; otherwise the new industry would rely on the conventional meat industry, turning any potential gain questionable.

Animal-free media compositions are usually proprietary information, although some known critical components are amino acids (mainly glutamine and arginine), several low concentration vitamins, inorganic salts, carbohydrates (mainly glucose and pyruvate) and cytokines. Cytokines, including growth factors such as FGF-2 and TGF-ß, and hormones such as insulin are responsible for the control of cell proliferation and differentiation, and may represent the most costly media components (Guan et al., 2021; Jairath et al., 2021; O'Neill et al., 2021). Alternative ingredients, such as yeast and microalgae extracts, have shown progress for mammalian cell medium supplementation (Okamoto et al., 2020; Spearman et al., 2016).

Alternative methods for cell cultivation have been studied to avoid adding complex media, aiming to lower production costs. An interesting method proposed by a Japanese company applied a set of bioreactors cultivating different cell lines, including liver cells, connected in series to a production bioreactor cultivating the cell-based meat. All bioreactors operated connected in perfusion mode, allowing for the growth hormones naturally produced in the first set of bioreactors to feed the production bioreactor. For this approach there is no complex media added (Yuki & Ikko, 2017).

Regarding scaling up, animal ingredients for the culture medium represent a major cost drawback. Also, the differentiation step seems more challenging than the cell proliferation phase. Differentiation in large scale presents different requirements and challenges depending on the goal in terms of final cell type. For example, myogenic differentiation may be more complex than adipogenic differentiation (Ng & Kurisawa, 2021; O'Neill et al., 2021).

Bioreactor types

The CM large-scale production in bioreactors is composed of two stages: (i) cell proliferation or expansion stage and (ii) cell differentiation and structuring stage (Table 1). The cell expansion step is easier to be scaled up in a semi-continuous (fed-batch) or continuous process, which reduces cell handling, prevents possible contamination and maximizes the productivity of cell proliferation. The most used bioreactors in cell expansion are stirred-tank bioreactors (STR), wave bioreactor, bubble column, airlift, packed and fluidized bed bioreactors (Ng & Kurisawa, 2021). The cell density obtained in the proliferation stage varies between 10^5 and 10^6 cells/ml with different cell lines (e.g., immortalized murine myoblast cells (C2C12), primary bovine myoblasts, primary rat myosatellite cells). Moreover, bioprocess conditions must be controlled to increase yields, such as dissolved oxygen, carbon dioxide, stirring speed, pH and temperature. Oxygen can be provided with natural or forced aeration, meeting the oxygen requirement of the cells through solubilization in the culture medium (Allan et al., 2019). A recent study showed a production of 5.053×10^5 cells/ml of bovine adipose-derived stem cells (bASCs) grown for 11 days in a 1 L stirred tank bioreactor with SoloHill Plastic microcarriers (Pall) in fed-batch mode (Hanga et al., 2021).

On the other hand, the cell differentiation step requires adaptation of scaffolds or microcarriers to bioreactors that support higher cell densities (e.g., hollow fiber bioreactor, fixed bed bioreactor) with 10^8 – 10^9 cells/ml. Scaffold or microcarriers are porous structures or matrixes which serve as a template for tissue formation by cell adhesion, allowing their proliferation and differentiation. These structures can be obtained from animal, plant, microbial or synthetic sources. Thus, scaffolds allow for the development of muscle, fat and connective tissues (Seah et al., 2021). They also offer a large surface to volume ratio when compared to a monolayer culture. To choose a good scaffold or microcarrier, some characteristics must be considered, such as edibility, biocompatibility, biodegradability, removability, bead-to-bead transference, low cost and also adequate physical, chemical and mechanical properties. Some examples of scaffolds or microcarriers used in CM production are Cytodex 1, Synthemax II, Cellbind, SoloHill Labs glass coated polymer, Cytodex-3, Biosilon, PP (Polypropylene), PS (Polysulfone), PES (Polyethersulfone), PLLA (Poly (L-lactic acid)) and cellulose triacetate hollow fibers (Allan et al., 2019). However, the development of better scaffolds or microcarriers to produce

Table 1. Bioreactors used in CM production.

Bioreactor type	Cell cultivation stage	Cell density	Advantages	Disadvantages	References
Stirred-tank bioreactor	Proliferation	10^5 – 10^6 cells/ml	· Good mixing and mass transfer. · Microcarriers can increase surface area without the need for a larger vessel.	· High shear stress. · Adhesion protein damage by aggregate dissociation · High energy consumption. · Foaming formation.	Allan et al. (2019), Bellani et al. (2020), Djisalov et al. (2021), Ng & Kurisawa (2021), Post et al. (2020)
Rotating wall/ wave bioreactor	Proliferation	10^5 – 10^6 cells/ml	· Low shear stress. · Low contamination. · Avoid energy costs of sterilization and time.	· Low cell density. · Disposable bags can be an environmental problem. · Scale limitation.	Allan et al. (2019), Bellani et al. (2020), Djisalov et al. (2021), Ng & Kurisawa (2021), Post et al. (2020)
Air-lift/bubble column	Proliferation	10^5 – 10^6 cells/ml	· Low shear stress. · Low contamination. · Low heat generation.	· Low homogeneity and mixing. · Difficult to scale-up. · Foaming formation.	Bellani et al. (2020), Li et al. (2020), Ng & Kurisawa (2021), Post et al. (2020)
Packed/Fixed/ fluidized bed bioreactor	Proliferation/ Differentiation	10^6 – 10^7 cells/ml	· High cell density. · Low shear stress. · Low operating cost.	· Low working volumes (about 100 L). · Low homogeneity, heat and mass transfer.	Allan et al. (2019), Bellani et al. (2020), Djisalov et al. (2021), Ng & Kurisawa (2021), Post et al. (2020)
Hollow fiber bioreactor	Differentiation	10^8 – 10^9 cells/ml	· High cell viability and density. · Cell differentiation stage with greater practicality. · Lower shear stress.	· Difficult cell harvesting. · Efficiency of cell harvesting may be limited by high microfiber and cell density.	Allan et al. (2019), Bellani et al. (2020), Djisalov et al. (2021), Ng & Kurisawa (2021), Post et al. (2020)

steak or fillets with organoleptic characteristics similar to traditional meat is still needed (Bodiou et al., 2020).

Recently, Memphis Meats, known as Upside Foods, published a perfusion apparatus technology which supports cell proliferation and differentiation in a cost-efficient system for growing cell sheets, and comprises a separator to recover the meat product from the substrate (Leung et al., 2021). However, there are no bioreactors designed specifically to produce CM. All proposed technologies have been based on the cultivation of animal or human cells in bioreactors to produce antibodies, recombinant proteins, vaccines, therapeutic proteins and tissues. The company Lonza Biologics PLC described in a published patent an STR bioreactor for mammalian cell cultivation with a working volume of 20 000 liters and at least two Rushton type impellers, which operate for about 15 days in batch or fed-batch mode, between 36 and 38 °C and at least a 10% seeding ratio with CHO (Chinese hamster ovary), NS0 (mouse myeloma) or hybridoma cell lines (M. Khan, 2017). Furthermore, Boehringer Ingelheim International GmbH proposed the use of a perfusion bioreactor connected to a continuous stirred tank bioreactor (CSTR) with at least between 14 and 30 days of cultivation using the cell lines CHO (Chinese hamster ovary), HEK-293 (human embryonic kidney 293), VERO (African green monkey kidney), NS0 (mouse myeloma), PER.C6 (human embryonic retinal cells), Sp2/0 (mouse myeloma), BHK (baby hamster kidney), MDCK (Madin-Darby canine kidney), MDBK (Madin-Darby bovine kidney) or COS (fibroblast-like cell lines derived from monkey kidney tissue) (Hiller et al., 2017).

Current market and patents overview

The market of CM is “fresh”. In August 2013, in London, Professor Mark Post of Maastricht University unveiled the world’s first cultured beef burger made from bovine stem cells. In 2016, the American company Memphis Meats announced the first lab-grown meat-ball, and in 2020 received considerable funding to expand their production in order to reach consumers (Memphis Meats, 2021). In 2017, the Israeli startup Aleph Farms was founded, joining expertise from the food company Strauss Group, the Technion – Israel Institute of Technology and the co-founder and CEO Didier Toubia. They announced their first cultivated beef steak in 2018 and have projects of meat for earth and space (Aleph Farms, 2021). In 2021, the first industrial plant to produce CM was inaugurated by the Israeli startup Future Meat. The plant has the capacity to produce 500 kg of meat products per day, and the company claims that their production process reduces greenhouse gas emissions in 80%, land use in 99%, water consumption in 96% and process time in 20x. The major concern related to the product, which is obviously the cost, was reduced in 1000x as compared to three years before, reaching a current cost of around US$ 10 for a piece of chicken breast (Minari, 2021). In parallel, many other companies are developing technologies and seeking approval to produce and commercialize CM throughout the world. We can mention the Dutch companies MosaMeat, funded by Professor Mark Post, and Meatable, the American Eat Just, the Brazilian BRF in partnership with Aleph Farms, the Chinese Avant Meats, the Singaporean Shiok Meats and the Israeli SuperMeat.

A patent search was performed to better understand the technological panorama on CM, and the results are presented in Figure 1. The first patent documents on this topic appeared in 1999 and, from then on, no significant increase in technological development was identified until 2019 (Figure 1a). In 2020 there was a huge rise in the number of patent publications, and this is likely to be the same for 2021. It is relevant to consider that there is a secrecy period of usually 18 months between the filing and the publication of a patent document. So, it is evident that most technologies related to CM are very recent and that the stage of technological maturity is probably not even close.

The fact that most patent documents (44%) were filed by companies shows that these new technologies are already transferred to the industrial sector, or were developed by companies themselves, though the cooperation with universities and research institutes was frequent among the publications, and the latter institutions accounted for 36% of patent filings (Figure 1b).

The top assignee was the pioneer Memphis Meats, with six patent documents, followed by Aleph Farms and Nanjing University, with four patent documents each. In third position there was the Chinese University of Jiangnan, and in fourth position, with two documents each, Avant Meats, the China Meat Research Centre, the American TUFTS College and the Israeli Yissum, the technology transfer company of the Hebrew University of Jerusalem (Figure 1c).

The first patent documents, filed in 1999 by van Eelen, van Kooten, Westerhof and Mummery, were entitled “Industrial scale production of meat from in vitro cell cultures” (WO9931222-A1) and “Industrial production of meat from in vitro cell cultures” (WO9931223-A1), and were related to the production of a meat product by culturing in vitro animal cells in a medium free of hazardous substances on an industrial scale, to produce three-dimensional animal muscle for human or animal consumption. The meat product comprised solidified cell tissue, obtained from muscle, somite, or stem cells.

From 2004 to 2009, four patent documents (US6835390-B1, US2005084958-A1, WO2006041429-A2 and SG155930-A1) were filed by Vein describing methods to cultivate animal muscle cells by exposing them to an electric or oscillating current and culturing them together with fat or cartilage cells or both in a support, variations of these methods, and the resulting meat products.

The most recent technologies developed by Memphis Meats involved extending the replicative capacity of a metazoan somatic cell population through decoupling retinoblastoma protein inhibition of cell division cycle advancement during replicative senescence, and maintaining telomerase activity (WO2017124100-A1); increasing the culture density of a metazoan cellular biomass by culturing in a cultivation infrastructure and inhibiting the HIPPO signaling pathway in the cellular biomass (WO2018208628-A1); increasing the cell density of a culture comprising metazoan cells, by introducing polynucleotide sequences encoding glutamine synthetase, insulin-like growth factor, and albumin into cells, and culturing the cells in a cultivation infrastructure (WO2019014652-A1); preparing a comestible meat product by applying non-human cells to a patterned texture substrate, growing these cells on this patterned texture substrate, and separating the meat product (WO2020243324-A1); increasing culture density and promoting anchorage-independent cell growth as an *ex vivo* method for producing edible metazoan cellular biomass, through culturing and activating a transcriptional enhanced associate domain protein (WO2021092587-A1); and an apparatus for preparing a meat product, comprising a substrate arranged within a cavity and nested surfaces

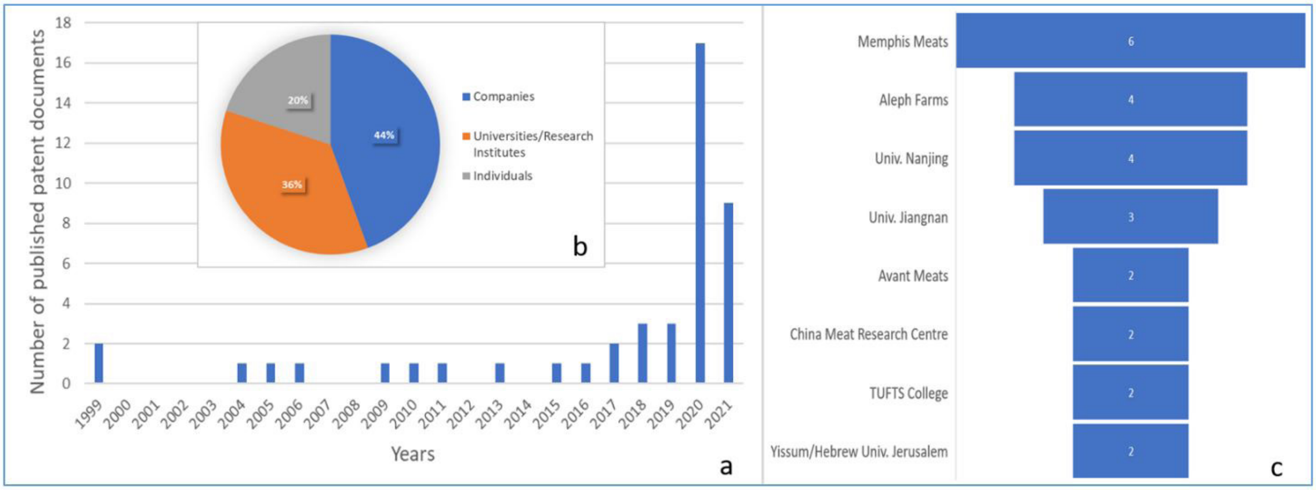


Figure 1. Patent documents related to animal cell meat. (a) Number of published patent documents per year; (b) Distribution of assignees by category; (c) Top-eight assignees and respective numbers of patent documents. Note: Synonyms for "animal cell meat" were [artificial or synthetic or in vitro or ex vivo or cell based or (cell)-culture(d) or vat grown or lab(oratory)-grown or animal free or slaughter free or animal cell or test tube or clean or engineered or cellular or in vitro cell(ular) cult(ure)] and [meat or beef or steak or flesh or shmeat or frankenmeat], and their orthographic variants. Synonyms were selected based on literature searches, especially on the manuscript (Bryant & Barnett, 2019). The names of the most important assignees were searched separately to find other possible documents. A total of 395 documents was found, of which 45 were related to animal cell meat. The search was performed in the Derwent Innovations Index database in the fields Topic (for keywords) and Assignee (for assignees' names), on August 3rd, 2021.

curved around a longitudinal axis and surface configured to support the growth of the meat product (WO2021102375-A1).

A patent document (CN112515113-A) filed by the China Meat Research Centre proposed the preparation of a cell culture meat product through 3D printing, after adding auxiliary material, flavor supplement and color supplement, into the cultivated meat raw material. The patent document WO2020230138-A1, filed by Aleph Farms, reports a serum-free liquid medium to cultivate non-human-animal-derived pluripotent stem cells, based on a combination of the growth factor bFGF, one additional growth factor and one small molecule selected from an inhibitor of the Wnt-P-catenin signaling pathway. New animal cell lines, culture media, cultivation systems and methods, bioreactors, animal cell compositions and non-human CM products were also protected in the patent documents.

Bioeconomy and sustainability

The current production of conventional meat and the expected rise of its consumption are of environmental concern, since meat production result in the emission of carbon dioxide (CO_2) (37 gigatons/year), methane (CH_4) (0.15 gigaton/year) and nitrous oxide (N_2O) (0.0065 gigaton/year), which are the major anthropogenic greenhouse gases (GHGs). Livestock alone is responsible for 15% of anthropogenic emission and it is, in fact, the major reason of anthropogenic methane emission (Godfray et al., 2018).

Conventional meat production does not only have an impact on GHG emission, but also in water resources and biodiversity. According to Mekonnen & Hoekstra (2012), nearly one third of the agriculture water footprint is connected with animal products. The authors claim that the rising production of conventional meat will increase even further the pressure on freshwater resources. Furthermore, Tilman et al. (2011) claim that manure lagoons are a great risk for surface and ground water, considering they carry different nutrients, toxins and pathogens. As for the impact on biodiversity, in South America pasture alone was the dominant driver of forest loss, being the cause of 71.2% of its deforest area from 1990 and 2005 (Sy et al., 2015). The conversion of natural habitats to agricultural land to produce grains and soy for livestock also affects biodiversity (Godfray et al., 2018). Thus, the advantages of CM are evident when compared to the cattle production, reaching 78 to 96% less greenhouse gases emission, 7 to 45% less energy use, 82 to 96% less water input, and 99% less land use (Tuomisto & Mattos, 2011). However, these values compared to poultry production reach similar effects in water and energy footprint, leaving land use as the most interesting advantage (Jairath et al., 2021).

Animal cell meat is actually one of the proposed methods for reducing environmental impacts caused by traditional and massive meat production. Tuomisto & Mattos (2011) used a life cycle assessment (LCA) analysis as a means to estimate the environmental impacts of large-scale *in vitro* meat production. The researchers concluded that to culture 1 ton of meat there was a requirement of 26-33 GJ energy, 367-521 m^3 water, and 190-230 m^2 land, with GHG emissions of 1900-2240 kg CO^2-eq. When compared to conventional meat production in Europe, CM required 7-45% less energy (except for poultry, which the in vitro cultivation demands more energy), 99% less land, and 82-96% less water, and GHGs production lowered by 78 to 96%. However, the authors make it clear that the calculations are based on many assumptions, which leads to a high uncertainty, and that new research and technologies must be developed in the field. Mattick et al. (2015a) discuss the results presented by Tuomisto and Mattos and report that the work was very significant contribution to both the in *vitro meat* and LCA literature. However, they also highlight that LCAs of emerging technologies, such as CM, face the challenge of the lack of availability of commercial-scale data, and should not be interpreted as definitive or conclusive. The development of new technologies also turns such a model less accurate throughout time. For those reasons, such studies could be both under or overestimating the impacts of CM. Chriki & Hocquette (2020) also claim that the advantages of CM for GHGs emissions are a matter of controversy, but still report the lesser demand of land.

In a more recent LCA, Mattick et al. (2015b) estimated an energy demand three times higher when compared to the prior estimates values using a stirred-tank bioreactor and cultivation medium based on corn. This result was mainly due to the basal medium production and the cleaning phase added to account. Mattick and collaborators also found that *in vitro* biomass will require more energy than livestock production. However, the authors claim that a level of uncertainty could suggest that the CM could be on the same level of energy consumption as beef production. Results were positive for risk of eutrophication analysis, which was considerably lower except for poultry, and for land use. This study highlights that different production parameters and practices added to the account can directly lead to great changes in estimate requirements for *in vitro* meat production, although the use of land is, in general, considered lower for this method. Therefore the development of new studies and technologies are of utmost importance to better inform stake-holders and society in terms of the real impact of CM to the environment (Mattick et al., 2015a).

Social aspects and public acceptance

As an extremely recent product in the food market, the CM is undergoing several studies in terms of public acceptance, legislation and potential change in consumption habits (Jairath et al., 2021). In order to commercialize it, the first main obstacle is the safety assessment and regulatory policy. As a food or raw material for food, it should present characteristics that offer safe and healthy consumption to the public, such as absence of pathogens and toxic compounds. Pertinent legislation also considers nutritional aspects, carbohydrate, protein and fatty acids profile and other characterization processes, not yet established for CM (Guan et al., 2021; Ng & Kurisawa, 2021; Post et al., 2020).

During the cultivation, animal cells are kept in a highly controlled environment inside a bioreactor, with a chemically defined culture media, initial sterilization process, antibiotics and selected cells (Bhat & Bhat, 2011a; Zhang et al., 2020). This control guarantees the complete or almost complete removal of animal diseases and pathogens, such as *Salmonella* and *Escherichia coli* (Bhat & Bhat, 2011a; Jairath et al., 2021), as well as heavy metals, pesticides and hormones conventionally applied during traditional meat production (Bhat & Bhat, 2011b; Bhat et al., 2015). Additionally, the resistant bacteria selection associated with the misuse of antibiotics during intensive animal farming can also be avoided (Chriki & Hocquette, 2020). However, the use of cultured cells rises concern related to genetic stability and tumorigenicity, and the long-term occurrence of mutations and loss of original protein profile should be constantly evaluated (Mohorčich & Reese, 2019).

The European Union was the pioneer in terms of regulatory policies for CM, inserting it as a novel food product together with new nanomaterials and polymers in food, effective from January of 2018 (Guan et al., 2021; Turck et al., 2016). The United States and Australia followed the tendency. In the case of the USA, both entities responsible for regulating food and meat, the Food and Drug Administration (FDA) and the US Department of Agriculture Food Safety and Inspection Service (USDA–FSIS), unified efforts to regulate the product, the first one responsible for the jurisdiction of the proliferation in bioreactor, and the second one for the post harvesting and labeling (Food and Drug Administration, 2019; Post et al., 2020). Australia and New Zealand included the product in the novel food frame, while waiting for premarket approval (Food Standards Australia New Zealand, 2017). Interestingly, Singapore was the first country to have a cultured chicken meat approved and commercialized in December 2020, with the “Good Meat” from the Eat Just start-up, followed by Israel, with another cultured chicken meat from the SuperMeat start-up (Listek, 2020; Poinski, 2020).

Currently, the culture media and the equipment required for cell growth represent a great economic obstacle. Initial formulations utilized fetal bovine serum as nutrient source, which is considerably expensive and generates ethical issues, since it is extracted from living bovines (Jairath et al., 2021; Zhang et al., 2020). If the perspective is to provide an alternative to conventional meat, it is vital to develop an inexpensive, eco-friendly and animal-free culture media, as well as a proper disposal of residues after harvesting the cells, in order to allow for a significant food demand to be covered (Bhat et al., 2015; Guan et al., 2021). The initial investment in research and industrial facilities limits the amount of people capable of developing this technology, while the conventional animal farm can be performed with basically land and pasture. It is interesting to highlight that in a potential scenario where the regular meat could be replaced by the CM, several farm workers would became unemployed, as the cultured cells require less labor (Post et al., 2020). However, on the other hand, the new industry tends to offer better-paid jobs, pushing for higher educational levels, to be less physically demanding and to involve more ability to control for comfortable working hours, as compared with the demanding schedules of some animal farming routines. These are examples of how the new industry may lead to improvements in human quality of life. In addition, all stages regarding meat processing activities will likely remain with the same job requirements, as the major change occurs in the first upstream stage of meat production. In addition, cell-based meat may require a diversification of plant agriculture, and it will certainly require the development of high volume of plant production to supply ingredients to a significant cell nutrition segment, which needs to thrive for CM to become a major component of the meat market. Finally, new job opportunities are likely to emerge within those companies supplying bioreactors and other required equipment. The major challenge regarding jobs seems to be the level of engagement with the new industry, as opportunities may off-set damages only in as much as the new industry becomes part of a country's industrial activities.

The last obstacles are related to the product characteristics and presentation, strongly determined by the sensorial traits and advertising. A controversial discussion is whether CM can be considered a vegetarian or vegan food (Alvaro, 2019; Guan et al., 2021). In this discussion, individual motivation and background may indicate whether a vegan person would eat CM or not. If the question is related to animal cruelty, suffering and slaughter, the CM significantly avoids animal suffering and completely avoids animal killing, as only small samples of tissue are removed through biopsy and the process can use only one animal for this purpose. Depending on the quality of the tissue maintenance, the cells may be cultivated for several generations, greatly avoiding long-term animal husbandry necessity and enhancing the original donors welfare (Alvaro, 2019; Bhat et al., 2015; Warner, 2019). Apart from the conceptual issues regarding vegetarianism and veganism, it is relevant to consider that the major public for CM is composed of those who regularly eat meat. The idea of producing real meat from cells instead of whole animals is a strategy that was designed to mitigate the increasing demand for meat within the next decades, a problem essentially centered in the meat consuming segment of society.

As the donation of cells is victimless, some completely new philosophical discussions may emerge, such as the production of meat from rare or exotic animals, or even from human beings, characterizing cannibalism (Bhat & Bhat, 2011a). Another important aspect is the religious affair. Both Jewish and Muslim have dietary laws to their meat products, called *Kosher* and *Halal* respectively. Although no conclusion was reached yet, it is crucial that the animal from which the cells are taken be considered *Kosher* or *Halal* for this public to accept its consumption (Chriki & Hocquette, 2020).

The organoleptic properties of the product, such as the texture, flavor and appearance, are also strong influencers in the consumer in the moment of buying (Ng & Kurisawa, 2021). The texture tenderness, juiciness and firmness are a result from different types of cell, fat and water concentration, as well as myofiber organization (Guinard & Mazzucchelli, 1996; Lonergan et al., 2010). The red-brownish color and opacity, on the other hand, are a result of different oxidation stages of myoglobin, and suggests the time of slaughter and quality of the cut (Hui et al., 2012), while the flavor profile is mainly determined by the fat and fatty acids stages of disposition, concentration and degradation (Calkins & Hodgen, 2007; Khan et al., 2015). To produce and simulate all such characteristics in a bioreactor environment is extremely difficult, hindering mainly the texture factor. Alternatives to approximate the cultured cell characteristics to original meat include 3D printing of the cells, tendon-gel-integrated bioprinting, scaffold design, biopolymers and nanoparticles addition, as well as the addition of colorants and flavoring compounds (Guan et al., 2021; Kang et al., 2021; Ng & Kurisawa, 2021). It is also relevant to mention nutritional aspects, such as vitamin and mineral content, which can be added to the product afterwards, together with preserving compounds to maintain stability (Ng & Kurisawa, 2021).

One last detail that may appear simple but determines the consumer approval is the name given to the product. Terms such as "animal-free meat" and "clean meat" appeal to the animal welfare and environmental characteristics, while "cultured meat" and "lab grown meat" refer to the production process. Depending on which term is used, region and background knowledge, the name can be associated with unnatural, disgust and strangeness by the public (Bryant & Barnett, 2019; Heidemann et al., 2020; Valente et al., 2019). However, this perception did not prevent respondents from showing predominantly a positive intention of consuming CM. The product terminology was not yet determined by any regulatory legislation, although there is a European proposal prohibiting the application of "steak, sausage, escalope, burger and hamburger" as the name for non-conventional meat products (Post et al., 2020). This proposal seems to lose momentum with the more recent signs by the European parliament, which have been positive in terms of labelling for alternative meats, such as for instance the voting later in 2020 for the keeping of veggie burger labels.

Challenges for the future of animal cell meat and further technological improvements

As shown in previous sections, real efforts and substantial technological improvements for the CM production in large scale and with sustainability, both in economic and environmental fields, are recent. In this sense, several scientific, technological and even political and social challenges are present in this growing and important theme. Some of the most important ones are briefly discussed below.

Regarding the animal cells used for cultivation, the obtainment of better primary cell lineages and their development remains an important concern. The currently developed techniques for CM production have been derived from tissue engineering, which include isolation, cell propagation and even co-culture of muscle and fat cells. This strategy is an important bottleneck in the production of steaks or fillets with characteristics similar to traditional beef, chicken and pork. Lowering the costs of culture media is also an important issue. Furthermore, genetic improvement of cells, gene editing or transfection may be applied to cell cultures, but could lead to rejection by consumers (Guan et al., 2021).

The bioreactors used in cell proliferation and differentiation still need to be improved and optimized to reduce costs in the production of CM. The trend in many biotech industries is single-use bioreactors (SUBs), which use disposable bags, decreasing the risks of contamination with clean-in-place (CIP). However, concerns about the final disposal of plastic bags and environmental impacts have been questioned, which may be offset by the lower energy consumption of this alternative (Allan et al., 2019).

On the other hand, the use of microcarriers that are inedible and non-biodegradable will require their separation from the final product, decreasing yields and increasing process steps. Thus, edible microcarriers appear to be promising in cell differentiation by reducing the stages of dissociation, separation and degradation; microcarriers that confer organoleptic properties or serve as nutrients for the cell may still be incorporated (Bodiou et al., 2020).

Furthermore, the development of a bioprocess without animal-derived sources (e.g., culture media, supplements, microcarriers) is in line with the proposal of alternative proteins, sustainability and animal welfare (Jairath et al., 2021; Seah et al., 2021). In summary, many challenges are presented. This is perhaps not surprising given the early stages of development for this radical innovation. The study of previous radical innovations shows a steep decline in production costs, due to the solution of main technological challenges, within the first 15 years of the emergence of a new technology (Arbib et al., 2021).

Conclusions

The CM has a great potential to replace several traits of conventional animal production, as well as provide several economic, social, environmental, and consumption habits change. In summary, for CM many technological improvements are still required to be developed. Economic and sustainability issues are a concern and intense research efforts in these fields seem warranted to support the best choices, as many positive or neagtive consequences of a transition to alternative meats are not deterministic, but will instead depend on our choices. Regulatory policy requires attention, guaranteeing proper discussion to provide for adequate and appropriate speed of establishment. Public acceptance needs further studies to be carefully evaluated and support best choices in all phases, especially the production and marketing stages. But the rising market and the growing number of patented processes coupled to the already established start-ups and enterprises operating in the field seem signals or at least promises that CM will be produced in industrial levels and globally commercialized in the years to come.

Considering all these factors, it is in the hands of the companies to decide whether to invest or not in this kind of technology, of governments to promote advantages by facilitating a positive entrepreneurial ecosystem for innovation to flourish within limits that are safe and beneficial for society at large, and it is in the hand of the consumers to choose this different product, influencing the adaption of the market to the respective level of public approval and it is in the hands of all stakeholders to find the best strategies to cope with the changes provided by this new food production strategy.

Conflict of interests

The authors declare that there are no conflicts of interest.

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