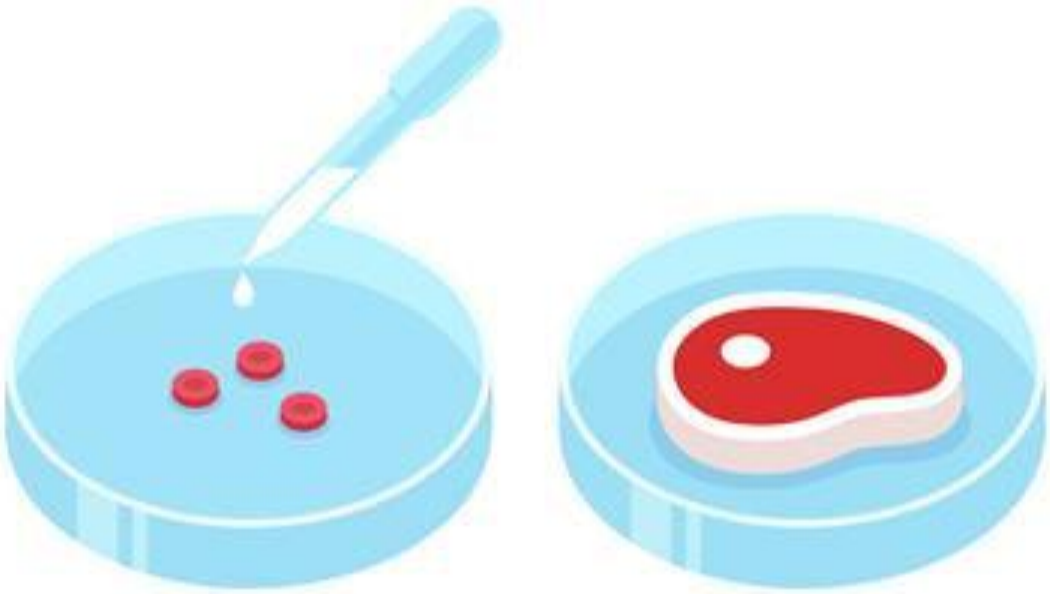
CELLUAR LIVESTOCK AGRICULTURE

Cellular Livestock Agriculture

Cellular livestock agriculture is an innovative approach to livestock production that utilizes cell cultures instead of whole animals. This method, also known as cellular agriculture, involves the cultivation of cells in a controlled laboratory setting, offering a sustainable alternative to traditional farming. The process begins with sourcing initial cells from a living animal through a small, minimally invasive biopsy, which are then selected for their ability to replicate extensively. Once a stable cell line is established, the cells are placed in a bioreactor, submerged in a nutrient-rich liquid, and encouraged to differentiate into specific types, such as muscle and fat cells, which are the primary components of meat. This method can produce products like cultured meat, leather, and other materials, providing a molecularly identical alternative to conventional meat production. Cellular agriculture is not only a way to produce animal proteins but also addresses the environmental impact and ethical considerations of animal farming. It aims to reduce greenhouse gas emissions, land and water use, and the reliance on large-scale animal farming. The technology is still in its early stages, but it holds the potential to revolutionize the way we produce animal products, offering a more sustainable and ethical alternative to traditional livestock farming.

Cellular Agriculture: Biotechnology for Sustainable Food

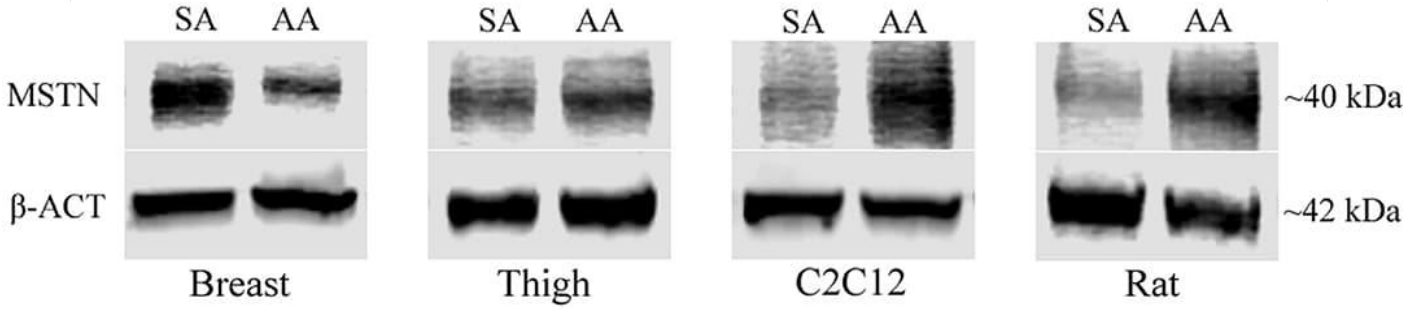


A Jewish Religious Perspective on Cellular Agriculture

Joel A. Kenigsberg, and Ari Z. Zivotofsky

Clean Meat is an emerging technology that promises to revolutionize the global food market. Alongside technological developments, the social impact of this innovation is being explored. Consumer acceptance will depend on multiple factors. For Orthodox Jews, the critical question

will be whether the new food can be defined as kosher. In the absence of an exact precedent in Talmudic case law, scholars have begun to examine which set of principles would govern the status of the new meat product. Traditionally, meat is permitted for kosher consumption only when it derives from a kosher species that has been kosher slaughtered in accordance with strict regulations. There is room to suggest that this same set of rules would determine the status of any product derived through cellular agriculture, and thus, the source cells would have to be extracted from a kosher species and only after kosher slaughter. Another approach would be to view the process as so different from the traditional growth of meat that it may be defined as kosher, even where traditional meat would not. Three determining factors will be: (1) the source of the original cells from which clean meat will be produced (animal species and cell type), (2) the nature of the growth medium, and (3) the exact nature of the process involved, which will determine whether the final product is considered a “new entity” or merely an “inheritance” of the starter cells. An authoritative ruling must be based on an in-depth appreciation of the scientific methods involved.



Ammonia Induces a Myostatin-Mediated Atrophy in Mammalian Myotubes, but Induces Hypertrophy in Avian Myotubes

Rachel A. Stern, and Paul E. Mozdziak

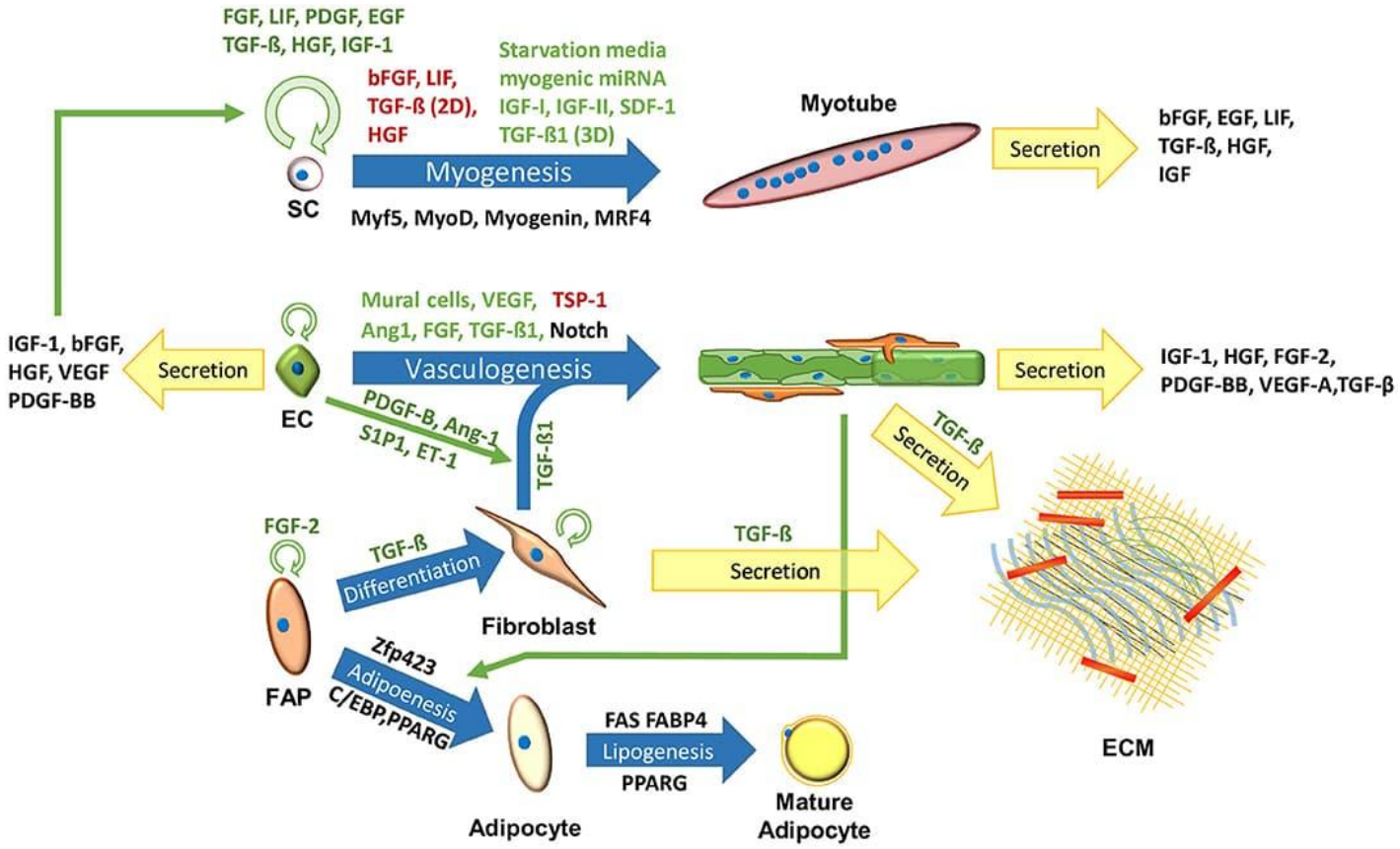
Ammonia, a byproduct of protein catabolism that is generally regarded as toxic, is processed by the liver for excretion. In diseases resulting in hepatic insufficiency, circulating ammonia levels increase dramatically, leading to secondary disorders. Sarcopenia, or loss of muscle mass, is commonly associated with hyperammonemia. In mammalian models of cirrhosis, increased myostatin is consistent, contributes to muscle autophagy, and reduces satellite cell activation and differentiation, whereas avian species show a positive myogenic response to ammonia. The objective of the study was to elucidate the effect of ammonia in chicken, mouse, and rat derived myotubes. Primary myoblasts were isolated from the pectoralis major (breast) and biceps femoris (thigh) of embryonic day 17 chicken embryos, and from the hindlimbs of 3-day-old rat pups. C2C12 cells were used for mouse myoblasts. Myotubes were exposed to 10 mM ammonium acetate (AA) or 10 mM sodium acetate (SA) for 24 h to determine myogenic response to ammonia. Relative expression of myostatin mRNA, determined by quantitative real-time PCR, was significantly higher in mammalian myotubes compared to chicken myotubes ($P < 0.001$). Western blot analysis of myostatin protein confirmed a significant increase in ammonia-treated rat myotubes, while chicken breast myotubes showed a significant decrease in myostatin ($P < 0.05$). Myotube diameter significantly increased in chicken breast and thigh cultures treated with ammonia, while diameter was significantly reduced in mouse and rat myotubes ($P < 0.05$). Intracellular glutamine is significantly higher in chicken thigh, but not breast, myotubes treated with AA compared to SA-treated myotubes ($P < 0.05$). To investigate fiber type differences in

ammonia metabolism, Western blot analysis of protein from AA and SA-treated myotubes was examined for fast and slow myosin heavy chain isoforms. AA treatment resulted in a higher ratio of fast to slow isoforms of myosin heavy chain in both types of chicken myotubes, while fast isoforms were decreased in AA-treated mouse and rat myotubes. These data demonstrate that chicken myotubes respond positively to ammonia while rodent myotubes respond negatively. Further, there is evidence that ammonia induces a fast fiber type shift in avian muscle, but a slow phenotype shift in mammalian muscle.

How Normal Meat Becomes Stranger as Cultured Meat Becomes More Normal; Ambivalence and Ambiguity Below the Surface of Behavior

Cor van der Weele, and Clemens Driessen

Although most people still behave like happy meat eaters, there are good reasons to think that many are in fact, ambivalent about meat. Following up on earlier findings, in this paper, we describe how, in focus groups, cultured meat triggered much discussion about meat, especially among older people. While young people wondered whether they would eat cultured meat products, older people thought about diet changes in a historical perspective and wondered if and how cultured meat might become a societal success. Beneath the surface of everyday behavior, in which they followed mainstream norms, many of our research participants harbored moral concerns and, in various ways, expressed an interest in collective change. Reflecting on the focus group discussions, we suggest, first, that appreciating the important role of ambivalence in processes of moral change requires rethinking relations between ambivalence and morality. Second, the entanglement of ambivalence with ambiguity increases the “fluidity” of such processes of change: when it is no longer clear what exactly meat is, the meanings and experiences of eating it also become unsettled. This has implications for thinking about morality in times of change. Studying consumer choices cannot do justice to processes of ambivalence and ambiguity below the surface of behavior. More generally, the idea that morality resides in making up our minds about clear moral choices gives way to the need to become skilled, collectively as well as individually, in dealing imaginatively with ambivalence and ambiguity.



Tissue Engineering for Clean Meat Production

Tom Ben-Arye, and Shulamit Levenberg

Increasing public awareness of foodborne illnesses, factory farming, and the ecological footprint of the meat industry has generated the need for animal-free meat alternatives. In the last decade, scientists have begun to leverage the knowledge and tools accumulated in the fields of stem cells and tissue engineering toward the development of cell-based meat (i.e., clean meat). In tissue engineering, the physical and biochemical features of the native tissue can be mimicked; cells and biomaterials are integrated under suitable culture conditions to form mature tissues. More specifically, in skeletal muscle tissue engineering, a plurality of cell types can be co-cultured on a 3D scaffold to generate muscle fibers, blood vessels, and a dense extracellular matrix (ECM). This review focuses on tissue engineering of skeletal muscle and the adjustments needed for clean meat development. We discuss the skeletal muscle structure and composition, and elaborate on cell types from farm animals that have the potential to recapitulate the muscle ECM, blood vessels, muscle fibers, and fat deposits. We also review relevant biomaterials, primarily for fabricating scaffolds that can mimic the intramuscular connective tissues, as well as gene expression studies on the biological pathways that influence meat quality.

Making Sense of Making Meat: Key Moments in the First 20 Years of Tissue Engineering Muscle to Make Food

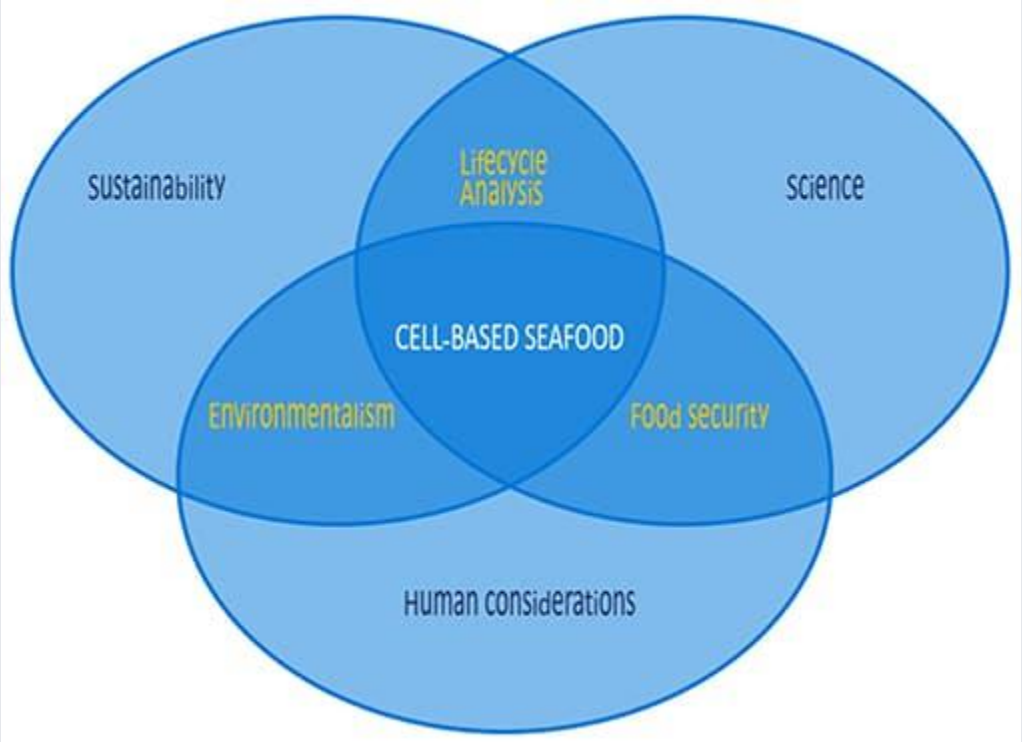
Neil Stephens, and Clemens Driessen

Cultured/clean/cell-based meat (CM) now has a nearly two-decade history of laboratory research, commencing with the early NASA-funded work at Touro College and the bioarts practice of the Tissue Culture and Art project. Across this period, the field, or as it is now more commonly termed, the "space," has developed significantly while promoting different visions for what CM is and can do, and the best mechanisms for delivery. Here we both analyse and critically engage with this near-twenty-year period as a productive provocation to those engaged with CM, or considering becoming so. This paper is not a history of the field, and does not offer a comprehensive timeline. Instead, it identifies significant activities, transitions, and moments in which key meanings and practices have taken form or exerted influence. We do this through analyzing two related themes: the CM "institutional context" and the CM "interpretative package." The former, the institutional context, refers to events and infrastructures that have come into being to support and shape the CM field, including university activities, conferences, third sector groups, various potential funding mechanisms, and the establishment of a start-up sector. The latter, the interpretative package, refers to the constellation of factors that shape or assert how CM should be understood, including the various names used to describe it, accounts of what it will achieve, and, most recently, the emergent regulatory discussions that frame its legal standing. Across the paper, we argue it is productive to think of the CM community in terms of a first and second wave. The first wave was more university-based and broadly covers the period from the millennium until around the 2013 cultured burger event. The second wave saw the increasing prevalence of a start-up culture and the circuits of venture capital interest that support it. Through this analysis, we seek to provoke further reflection upon how the CM community has come to be as it is, and how this could develop in the future.

Bioprocess Design Considerations for Cultured Meat Production With a Focus on the Expansion Bioreactor

Scott J. Allan, and Marianne J. Ellis

Cultured meat, as a cellular agriculture product, utilizes tissue engineering techniques and consequently faces not only cell culture challenges but also scale-up limitations. To ensure cultured meat is financially viable, efficient bioprocess design for scale-up is required. In this mini-review, we focus on the design of the expansion bioreactor, and put it in context of the entire bioprocess by providing an overview of the upstream and downstream process considerations. As a full-scale cultured meat bioprocess is still hypothetical, we include a review of the key factors and fundamental cell biology parameters required as input data for the design of a process with a product that is not only viable but price-competitive. This review highlights the vital aspects of a cultured meat bioreactor design that are often overlooked when parallels are drawn against fermentation processes such as brewing or recombinant protein production in the pharmaceutical industry. Practical application and awareness of the concepts presented here will enable more accurate estimation of the production expenses and raw material requirements. This will form a basis for both further academic research and the design of industrial-scale processes in the field of cultured meat and the wider field of tissue engineering-based cellular agriculture.

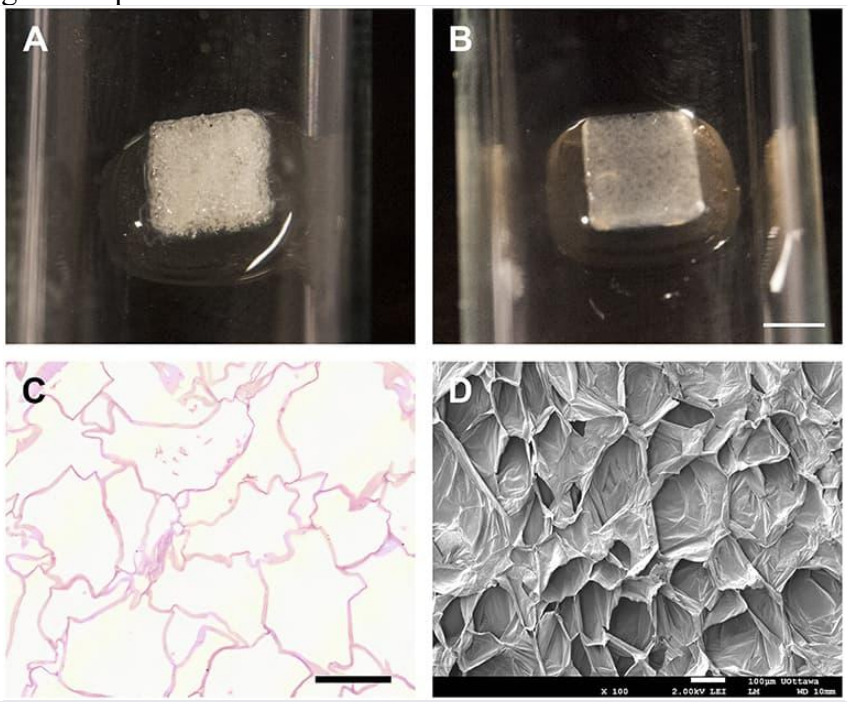


Cell-Based Fish: A Novel Approach to Seafood Production and an Opportunity for Cellular Agriculture

Natalie Rubio, and Kate Krueger

Cellular agriculture is defined as the production of agricultural products from cell cultures rather than from whole plants or animals. With growing interest in cellular agriculture as a means to address public health, environmental, and animal welfare challenges of animal agriculture, the concept of producing seafood from fish cell- and tissue-cultures is emerging as an approach to address similar challenges with industrial aquaculture systems and marine capture. Cell-based seafood—as opposed to animal-based seafood—can combine developments in biomedical engineering with modern aquaculture techniques. Biomedical engineering developments, such as closed-system bioreactor production of land animal cells, create a basis for the large-scale production of marine animal cells. Aquaculture techniques such as genetic modification and closed system aquaculture have achieved significant gains in production that can pave the way for innovations in cell-based seafood production. Here, we present the current state of innovation relevant to the development of cell-based seafood across multiple species, as well as specific opportunities and challenges that exist for advancing this science. The authors find that the physiological properties of fish cell- and tissue- culture may be uniquely suited to cultivation *in vitro*. These physiological properties, including tolerance to hypoxia, high buffering capacity, and low-temperature growth conditions, make marine cell culture an attractive opportunity for scaled production of cell-based seafood; perhaps even more so than mammalian and avian cell cultures for cell-based meats. This opportunity, coupled with the unique capabilities of crustacean tissue-friendly scaffolding such as chitosan, a common seafood waste product and mushroom derivative, presents promise for cell-based seafood production via bioreactor cultivation. To become fully realized, cell-based seafood research will require more understanding of fish muscle cell and tissue cultivation; more investigation into serum-free

media formulations optimized for fish cell culture; and bioreactor designs tuned to the needs of fish cells for large-scale production.



Scaffolds for 3D Cell Culture and Cellular Agriculture Applications Derived From Non-animal Sources

Santiago Campuzano, and Andrew E. Pelling

For decades, two-dimensional cell culture has been regarded as a major tool in cellular and molecular biology due to its simplicity, reproducibility, and reliable nature. However, it is now recognized that 2D cell culture underrepresents the *in vivo* environment of living cells. The development and use of 3D scaffolds and biomaterials provide researchers the ability to more closely mimic the *in vivo* environment. However, many biomaterials are of animal origin, leading to variability, environmental, and ethical concerns. Here we present three animal-free scaffolds: decellularized plant tissue, chitin/chitosan and recombinant collagen. Decellularized plant tissue provides a wide array of structures with varying biochemical, topographical, and mechanical properties; chitin/chitosan-based scaffolds have shown synergistic bactericidal effects and improved cell-matrix interaction; and lastly, recombinant collagen has the potential to closely resemble native tissue, as opposed to the other two. These benefits, alongside potential scalability and tunability, open the door to applications beyond the biomedical realm, such as innovations in cellular agriculture and future food technologies.

A Survey of Consumer Perceptions of Plant-Based and Clean Meat in the USA, India, and China

Christopher Bryant, and Brian Tse

Recent years have seen increasing interest in research on consumer acceptance of clean meat. Whilst some consumers are enthusiastic about the prospect of reducing the health risks, environmental harms, and animal welfare implications associated with conventional meat production, others have concerns about the product's taste, price, safety, and naturalness. Some

evidence suggests that acceptance of clean meat will vary substantially across cultures, though there is currently a lack of quantitative research in Asia and country comparisons on this topic. Both are likely to be important areas given the forecasted increase in meat consumption in developing countries. Participants (n = 3.030) were recruited through the research panel CINT to take an online questionnaire about clean meat and plant-based meat. The participants were representative of China, India, and the U.S. in terms of age and gender, though participants in India and China were disproportionately urban, high-income, and well-educated. As well as clean meat, participants were asked about plant-based meat, a conceptually similar product with similar potential to displace demand for conventional meat. They also answered the Meat Attachment Questionnaire and the Food Neophobia Scale. We compared these variables between countries and used regression models to identify which demographic and attitudinal factors predicted purchase intent toward both products. We found significantly higher acceptance of clean and plant-based meat in India and China compared to the USA. We also found significantly higher food neophobia and significantly lower meat attachment in India compared to China and the USA. We identified several demographic patterns of clean and plant-based meat acceptance as well as which beliefs were important predictors of acceptance within each country. In particular, higher familiarity predicted higher acceptance of plant-based and clean meat across all countries. We found high levels of acceptance of clean meat in the three most populous countries worldwide, and with even higher levels of acceptance in China and India compared to the USA. These results underline the importance of clean meat producers exploring new markets for their products, especially as meat consumption in developing countries continues to rise.

Possibilities for Engineered Insect Tissue as a Food Source

Natalie R. Rubio, Kyle D. Fish, Barry A. Trimmer, David L. Kaplan

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Due to significant environmental concerns associated with industrial livestock farming, it is vital to accelerate the development of sustainable food production methods. Cellular agriculture may offer a more efficient production paradigm by using cell culture, as opposed to whole animals, to generate foods like meat, eggs, and dairy products. However, the cost-effective scale-up of cellular agriculture systems requires addressing key constraints in core research areas: (1) cell sources, (2) growth media, (3) scaffolding biomaterials, and (4) bioreactor design. Here, we summarize work in the area of insect cell cultures as a promising avenue to address some of these needs. We also review current applications of insect cell culture and tissue engineering, provide an overview of insect myogenesis, and discuss various properties of insect cells that indicate suitability for use in food production systems. Compared to mammalian or avian cultures, invertebrate cell cultures require fewer resources and are more resilient to changes in environmental conditions, as they can thrive in a wide range of temperature, pH and osmolarity conditions. Alterations necessary for large-scale production are relatively simple to achieve with

insect cells, including immortalization, serum-free media adaptation, and suspension culture. Additional benefits include ease of transfection, nutrient density, and relevance to seafood organisms. To advance insect-based tissue engineering for food purposes, it is necessary to develop methods to regulate the differentiation of insect cells into relevant cell types, characterize cell interactions with biomaterials with an eye toward 3D culture, design supportive bioreactor systems, and quantify nutritional profiles of cultured biomass.

Introduction

Animal Agriculture and Food Sustainability

The Intergovernmental Panel on Climate Change of the United Nations released a recent report stating that in order to prevent global warming from increasing above 1.5°C, a goal that could prevent the severity of natural disasters correlated with a 2.0°C increase, anthropogenic greenhouse gas (GHG) emissions need to decrease 45% by 2030 (Intergovernmental Panel on Climate Change, 2018). In 2013, the Food and Agriculture Organization (FAO) of the United Nations assessed animal agriculture to be responsible for 14.5% of anthropogenic GHG emissions (Gerber et al., 2013). The FAO identified the principle sources of these emissions to be feed crop production (45%), enteric fermentation (39%), and manure management (10%) (Gerber et al., 2013). Mitigation strategies include optimizing current food systems (e.g., improving breeding, animal health, and feed) and developing new products with lower environmental footprints.

Researchers and entrepreneurs have proposed numerous alternative food systems to supplant conventional meat, eggs, and dairy, including transgenic animals (Thornton, 2010), plant-based analogs (Smetana et al., 2015), and insect farming (van Huis and Oonincx, 2017). In addition to combating climate change, the projected benefits of these novel approaches include improved food safety, nutrition, and animal welfare. However, each of these approaches has significant drawbacks. Although plant-based meat analogs are relatively affordable and sales in the United States are rising each year (Plant Based Foods Association, 2018), the products face a number of limitations: (1) primary consumers are vegetarians/vegans, not omnivores, (2) for analogs that aim to fully emulate animal products, the slightest discrepancies in taste/texture/behavior can produce an “uncanny valley” effect (Hoek et al., 2011), (3) plant products generate waste unless all material is utilized and some crops (e.g., almonds, walnuts, rice) have water footprints equivalent to meat and poultry (Mekonnen and Hoekstra, 2011). Transgenic animals are also being pursued; however, they often only address a single issue, such as reduced methane production or disease resistance (Clark and Whitelaw, 2003; Bruce et al., 2013).

Entomophagy is another proposed solution to combat the detrimental effects of intensive animal farming. The suggested environmental benefits of insect farming include lower greenhouse gas emissions, lower water requirement, lower space requirement (e.g., vertical farming), rapid growth and reproduction rate, and high food conversion efficiency (largely due to differences in edible weight fraction, crickets and beef are 80 and 40% edible, respectively) (van Huis et al., 2013; Tabassum-Abbasi, 2016). Cricket flour is gaining some traction in the United States due to the proposed benefits of both sustainability and nutrition, as many insect species are shown to be high in protein, healthy fats, and minerals (Rumpold and Schlüter, 2013; Mason et al., 2018).

However, due to food neophobia, it will likely be a challenge to achieve mainstream acceptance for insect consumption in Western cultures, despite the prevalence of this practice in other parts of the world (Hartmann et al., 2015; Verbeke, 2015). Furthermore, due to innate taste and texture differences, foods from whole insects may not satisfy meat and poultry cravings (Caparros Megido et al., 2016).

To reduce the consequences associated with resource-intensive animal agriculture and overcome the challenges of existing alternatives, it is important to continue identifying and pursuing new food system innovations with potential for transformative impact.

Cellular Agriculture

Cellular agriculture is the emerging industry and field of study associated with producing agricultural commodities (e.g., meat, dairy, leather) via cells as opposed to whole animals. Such products have the potential to address industry issues and consumer needs not met by other alternative foods. By removing the animal from the production process, advocates of the technology suggest that meat produced via cellular agriculture (i.e., cultured meat) will be safer, more humane, and better for the environment relative to conventional products produced through animal farming (Bhat et al., 2015). For example, a 2014 life cycle assessment estimated that compared to farmed meat, cultured meat could require up to 45% less energy, 96% lower greenhouse gas emissions, 99% lower land use, and 96% less water (Tuomisto and Teixeira de Mattos, 2011). A separate study concluded that while resource and land inputs could be less for cultured meat than for conventional meat, energy requirements could be higher (Mattick et al., 2015). The abridged process for cultured meat production involves (1) procuring cells, (2) proliferating the cells in bioreactors, (3) adhering the cells to scaffold systems, and (4) promoting differentiation into muscle and fat tissues (Arshad et al., 2017).

Cultured meat technology is currently in the early stages of research and development. Cells from various species, primarily bovine, porcine, and avian, have been targeted for cultured meat production (Genovese et al., 2017; Verbruggen et al., 2018). However, cells obtained from less obvious species may prove useful in overcoming the ongoing technical challenges restricting cellular agriculture advancement and scale-up, including high media costs and the necessity of adherent culture conditions for many cell types. In particular, insect cells have several qualities that suggest the feasibility of large-scale production, such as ambient growth conditions, low media requirements, and straightforward adaptation to suspension and serum-free culture (Neermann and Wagner, 1996; Donaldson and Shuler, 1998; Lynn, 2001; Ikonomou et al., 2003; Mitsuhashi and Goodwin, 2018). Pursuing research and development for insect cell-based foods may be a powerful means of generating new sustainable food products and accelerating the field of cellular agriculture.

Insect Cell Culture

History of Insect Cell Culture

Early projects involving insect cell culture included monitoring the growth and development of spermatozoa from the gypsy moth, and examining the progression of wilt disease with insect

hemocytes (Glaser and Chapman, 1912; Goldschmidt and Wilhelm, 1915). In 1962, the first continuous insect cell lines were established, setting the stage for insect cell culture to become a staple of entomological research (Grace, 1962). These developments and initial uses of insect cell culture were followed in the rest of the twentieth century by the establishment and application of hundreds of insect cell lines from a variety of orders, which have been reviewed elsewhere (Lynn, 2007; Smagghe et al., 2009).

Metabolism and Culture Conditions

Compared to mammalian cells, insect cells are capable of tolerating a broad range of environmental conditions (Akiyama et al., 2013) (see Table 1). The ability to proliferate and maintain physiological function in variable pH, temperature, and oxygen conditions makes insect cell culture an attractive system for food production. Robustness across environmental variables allows different culture conditions to be tuned for maximum efficiency (Reuveny et al., 1993). Insect cells can be grown at scale at room temperature, conserving the energy input generally required for incubating mammalian cells. Many insect cell lines can also be grown without supplementing carbon dioxide, further simplifying culture systems and increasing economic viability relative to mammalian cells (Irons et al., 2018).

Factor	Insect cells	Mammalian cells	References
Temperature	20–32°C	30–39°C	Sisken et al., 1965; Agathos et al., 1990; Reuveny et al., 1993; Furukawa and Ohsuye, 1998; Gotoh et al., 2004; Vergara et al., 2014
pH	6.0–7.0	6.8–7.8	Grace, 1962; Ceccarini and Eagle, 1971; Wong et al., 1992; Baryshyan et al., 2014; Vajrala and Murhammer, 2016; Mackenzie et al., 2018
Osmolality	340–390 mosm/kg	290–330 mosm/kg	Vaughn, 1968; Waymouth, 1970; Ozturk and Palsson, 1991; Zhang et al., 1994
Contact inhibition	No	Yes	Todaro et al., 1965; Agathos, 1991; Leontieva et al., 2014
Substrate detachment method	Mechanical	Enzymatic	Weiss, 1963; Lynn, 2002

Table 1. Comparison of insect and mammalian cell growth conditions and methods of culture.

Lactic acid buildup due to cellular metabolism is problematic in mammalian cell cultures, as the accumulation of lactic acid and the associated drop in pH reduce proliferation (Konakovsky et al., 2016). Insect metabolic pathways mitigate this issue by circumventing the production of lactate from glucose (Ferrance et al., 1993; Neermann and Wagner, 1996). These properties enable maintenance of high rates of proliferation that are beneficial for mass production of cells. Furthermore, total glucose consumption has been reported to be lower in insect cells than mammalian cells, indicating potential for cost savings through reduced media usage (Neermann and Wagner, 1996).

Current Applications

Recombinant Protein Production

In addition to their utility in insect physiology and disease research, insect cell culture has emerged as a powerful tool for biomanufacturing. By coupling scalable cell culture with engineered baculovirus expression vector (BEV) systems, insect cells can be leveraged to generate large quantities of viral or protein products (Ikonomou et al., 2003). Current

applications of this technology include the production of bio-insecticides, therapeutics, vaccines, and recombinant proteins. Sf9, Sf21, and High Five are three of the most commonly used cell lines for industrial biomanufacturing applications, and are favored over mammalian cell lines for their robustness and production efficiency (Drugmand et al., 2012). Glybera and Flublok are examples of human therapeutics produced from insect cell culture and BEV technology (van Oers et al., 2015).

Bioactuators

Insect muscle culture is prominent in the field of soft robotics. Researchers have constructed small-scale robots or "bio-bots" from both mammalian and invertebrate biomass (Ricotti et al., 2017). Insect-derived bio-bots may be superior to bio-bots constructed from mammalian tissues or cells because insect tissue is tolerant to a wide range of environmental conditions. For example, *Ctenoplusia agnate* dorsal vessel tissues (DVT) were found to contract *in vitro* at 5–35°C (Akiyama et al., 2008c), and *Manduca sexta* muscle cells maintain contractions at 15–37°C and at pH values ranging from 5.5 to 7.5 (Baryshyan et al., 2014). Furthermore, insect muscle tissue can contract without external stimuli or media refreshment for extended periods (18, 30, 90, and 250 days have been reported for various species) (Akiyama et al., 2008a,b, 2009). Although the majority of experiments use excised DVT for force generation, select studies have used muscle cell cultures dissociated from either DVT or embryos (Akiyama et al., 2008b; Shimizu et al., 2010; Baryshyan et al., 2014). Because of differences in the size and measurement methods between systems, it is difficult to compare the forces generated by cultured muscles, but portions of the insect DVT produce between 4.7 and 20 μN (Akiyama et al., 2009, 2012a), and similarly sized constructs from neonatal rat cardiomyocytes produce 1.7 μN (Horiguchi et al., 2009). Cultured *M. sexta* muscle cells can generate approximately 2 kPa of stress (Baryshyan et al., 2014), which is similar to that estimated for cultured rat myocytes (Dennis et al., 2001). When directly comparing contraction capacity via index of movement (IOM) of C2C12s and primary *M. sexta* myotubes, *M. sexta* myotubes contracted throughout the entire measured timeframe (23 days) while C2C12s stopped contracting after day 9 (day 0 is defined as the day contractions began). Moreover, IOM measurements were typically higher values for *M. sexta* myotubes than the C2C12s (Baryshyan et al., 2012). Bioactuator devices have increased in complexity over time, from substrate-free tissue contractions and micro-pillar arrays to autonomous micro-robots, atmospheric-operable micro-tweezers and pumps (Akiyama et al., 2008b,c, 2012a,b, 2013; Tanaka et al., 2017).

Insight gained from biomanufacturing and bioactuator research can be translated to insect muscle cultured for food applications. For instance, efforts to improve the efficiency and cost-effectiveness of recombinant protein production through insect cell-based biomanufacturing have led to the development of bioreactor systems and low-cost media formulations relevant to insect cellular agriculture. Furthermore, bioactuator technology may be applicable since cultured muscle may require contractile stimulation to increase differentiation, affect protein expression, and impact texture (Datar and Betti, 2010). Uesugi et al. (2016) used insect-based bioactuator devices derived from the final stage larva of *Thysanoplusia intermixta* to compare the *in vitro* effects of tensile, thermal, electrical, and chemical stimuli. They found that tensile, thermal, and electrical stimuli could regulate contractile force, while thermal and chemical stimuli could regulate contractile frequency. A separate study concluded that chemical stimuli via crustacean cardioactive peptide could increase contractions of *C. agnata* DVT by 5.6-fold and may be

superior to electrical and thermal methods, which can be detrimental to the cells (Akiyama et al., 2008a). Yet another study successfully stimulated *Drosophila melanogaster* DVT via optogenetic engineering and light pulses of 2 Hz, suggesting optogenetics as a promising low-cost and non-invasive control strategy (Suzumura et al., 2011).

Insect Muscle Development

To pursue the development of insect cell-based food products, it is essential to understand the growth and development of relevant cell types (primarily muscle and fat) in their endogenous context. *D. melanogaster* and *M. sexta* are two of the most frequently studied insects for myogenesis research, and muscle formation is reported to be similar in the two organisms (Duch et al., 2000; Schnorrer et al., 2010; Ayme-Southgate et al., 2015). Here, we review the processes involved in embryonic and adult myogenesis in *D. melanogaster* (see Figure 2). As various cell types and species of insects are explored for cellular agriculture applications, additional research will be necessary to characterize the unique biological pathways responsible for *in vivo* cell development and *in vitro* culture.

Embryonic Myogenesis

In *D. melanogaster*, somatic larval muscle is derived from groups of mesodermal cells expressing high levels of the transcription factors Twist and Sloppy Paired. Within each group, one cell becomes a myogenic progenitor cell and the rest becomes fusion-competent myoblasts. The myogenic progenitor cells give rise to muscle founder cells, each of which fuses with 4–25 of the surrounding fusion-competent myoblasts to form larval muscle. Myogenic progenitor cells can also generate quiescent adult muscle precursor cells (Gunage et al., 2017). Fusion is facilitated through the membrane adhesion proteins Dumbfounded (on founder cells) and Sticks-and-Stones (on fusion-competent myoblasts) (Schnorrer and Dickson, 2004). Many of the muscles formed during embryogenesis are remodeled during metamorphosis, triggered by dynamic hormone levels (Baryshyan et al., 2012).

Adult Myogenesis

During adult muscle development in *D. melanogaster*, previously quiescent adult muscle precursor cells are activated and proliferate, either *de novo* (dorsal ventral flight and abdominal muscles) or guided by preexisting larval muscle (dorsal longitudinal flight muscles). Like in embryonic myogenesis, the majority of adult muscle precursor cells differentiate into fusion-competent cells, and a subgroup becomes founder cells. The myofibers form a myotendinous junction with tendon cells before differentiating into contractile muscles. Adult muscle precursor-derived muscle satellite cells have also been reported to respond to injury in *D. melanogaster*, characterized by expression of the transcription factor Zfh1 (Gunage et al., 2017). Similar mechanisms are reported in other insect species. In *M. sexta*, the dorsal longitudinal flight muscles also develop from the fusion of myoblasts with preexisting larval muscle, and motorneuronal innervation is reported to be important for the correct formation of the adult muscle fibers (Duch et al., 2000). In both species, muscle development is influenced by ecdysteroid levels (Weeks and Truman, 1986; Truman et al., 1994).

Insect Cells for Cellular Agriculture

In 2001, insect cell culture was proposed as a production system for human food (Verkerk et al., 2007). However, little progress on the subject has been made in the past 17 years. The recent surge in public attention and innovation in the field of cellular agriculture marks an opportune moment to revisit insect cells as a nutritional source. As the technical hurdles constraining mammalian cell-sourced cultured meat development have come into focus, the potential utility of insect cells has become increasingly apparent. Many of the same characteristics that make insect cells suitable for use in biomanufacturing and soft robotics also make them promising candidates for efficient, scalable food production.

Cell Sources

Muscle Cells

Obtaining a stable and scalable source of insect muscle and fat cells is an important challenge in the effort to engineer insect tissues for food. Despite the emergence of insect cell culture as an important biotechnology, minimal attention has been given to the establishment of muscle cell lines. However, there are previous reports of primary and immortalized myoblasts from *D. melanogaster*, and of contractile myotubes from *M. sexta* (Bernstein et al., 1978; Baryshyan et al., 2012; Dequéant et al., 2015). These protocols are valuable in providing a means of investigating relevant aspects of insect muscle cell biology *in vitro*. However, for production purposes, stable lines of muscle progenitor cells capable of extended proliferation and eventual differentiation is important (Kadim et al., 2015).

Insect muscle cells have been isolated and cultured from *D. melanogaster* and *M. sexta* embryos and from *M. sexta* stage 2 pupae (Luedeman and Levine, 1996; Simcox et al., 2008; Baryshyan et al., 2012). For *D. melanogaster* embryonic isolation—embryos laid overnight at 17°C are sterilized with 50% bleach and rinsed with a detergent solution before homogenization with Schneider's medium supplemented with 10% fetal bovine serum. Embryonic-cell-containing supernatant is centrifuged and rinsed before plating roughly 100 µL of the cell pellet into three T-25 culture flasks. Cells are incubated at 22°C, and the media is refreshed every other week (Simcox et al., 2008). For *M. sexta* embryonic isolation, −19 to 22 h old embryos are collected and sterilized with 25% bleach and rinsed with EGTA-media before homogenization. The supernatant is centrifuged and plated at 5 embryos per cm^2 in a L15/Grace ‘s-based media with 12% fetal bovine serum, EGTA, and other supplements. After 2 h, non-adherent cells are removed, the plates are refreshed with EGTA-free and cells are incubated at 26°C (Baryshyan et al., 2012). For adult *M. sexta* isolations, pupae are sterilized, and the leg muscles are dissected and digested with collagenase-dispase. The tissue is centrifuged and rinsed with L15/Grace 's-based serum-free media before plating at one leg per culture dish (Luedeman and Levine, 1996). Both primary embryonic and pupae myoblasts are able to proliferate and differentiate compared to genetically immortalized adult muscle precursor cells from *D. melanogaster*, which exhibit strong proliferation but limited differentiation capacity (Dequéant et al., 2015; Rubio et al., 2019). Limited differentiation in immortalized insect muscle cells may be due to the lack of supporting cell types present in primary cultures, as there is some evidence that insect myoblasts require direct contact with neurons to fully mature (Luedeman and Levine, 1996; Gunage et al., 2017). More research is needed to determine if the relationship between insect age and

proliferation capacity is similar to trends in mammalian cell types, in which cell multiplication decreases with an increase in animal age (Schultz and Lipton, 1982).

The ability to control the proliferation and differentiation of cultured muscle cells is also important for large-scale production. While the hormonal pathways involved in insect muscle development are not as well elucidated as those in humans or other animal species, valuable progress has been made in this area. Specifically, muscle remodeling has been shown to be under the control of steroid hormones known as ecdysteroids (Hegstrom and Truman, 1996). In *M. sexta*, myoblast proliferation is stimulated by moderate levels of 20-hydroxyecdysone and inhibited by adjustment of the concentration beyond a critical threshold (Champlin et al., 1999). High levels of ecdysteroid can be used to induce proliferative arrest and differentiation, an effect that is inhibited by the presence of methoprene, a juvenile hormone analog (Champlin et al., 1999). These hormonal controls of the differentiation pathway can be exploited to regulate cell development during production.

Fat Body Cells

In cellular agriculture, fat is important for both taste and nutrition. Many insect species contain high amounts of essential fats like omega-3 and omega-6 fatty acid (van Huis et al., 2013). Apart from lipids, insect fat body tissue also contains carbohydrates and proteins (Hoshizaki and Chapman, 2012). To produce nutritious and palatable food products, it will be important to culture insect fat tissue alongside muscle. Insect fat body tissue is analogous to adipose and liver tissue in vertebrates in that fat body cells both store and metabolize energy and nutrients *in vivo* (Hoshizaki and Chapman, 2012). Fat body cells are also beneficial for *in vitro* culture of other insect cells. Nutrient storage and release by fat cells may increase muscle cell survival and contraction *in vitr*o for month-long durations without media refreshment (Baryshyan et al., 2012). Similarly, fat body cell conditioned media may promote *in vitro* embryonic development (Ferkovich et al., 1991). Fat body was one of the first insect tissue types to be cultured *in vitro* within the study of protein synthesis (Raikhel et al., 1997). Fat body cells synthesize important proteins, including juvenile hormone binding protein, vitellogenin, and yolk protein precursors (Nowock et al., 1975; Wyatt, 1988).

Multiple publications have detailed insect fat body cell isolation procedures. Most protocols use late-stage larval animals and sterilize the surface of the animal with ethanol or sodium hypochlorite (Mitsuhashi, 1981; Philippe, 1982). Fat body tissue is excised from the abdomen and minced in either buffer solution or culture media (Nowock et al., 1975; Mitsuhashi and Inoue, 1988; Kishimoto et al., 1999; Lynn, 2001). Most protocols cultured the explants without digesting the tissue, although one study used Dispase I to dissociate the cells (Kishimoto et al., 1999). A wide variety of culture media formulations have been used for fat body cell culture, including basal media with or without antibiotics and with or without fetal bovine serum (Mitsuhashi, 1981; Philippe, 1982; Mitsuhashi and Inoue, 1988; Kishimoto et al., 1999). Most protocols incubated the cells around 25–27°C and refreshed media once every 1–2 weeks (Mitsuhashi, 1981, 1984; Philippe, 1982; Mitsuhashi and Inoue, 1988). Passaging can often be performed via gentle mechanical disruption, contrary to the enzymatic methods commonly used with vertebrate cells (Lynn, 2002). While most cell lines were cultured as adherent, one study established a cell line from *S. seriatopunctata* that grew in suspension (Mitsuhashi, 1984). Fat body tissue cultures often contain heterogeneous cell types (see Table 2), the morphologies of

which have been described in the literature (Mitsuhashi, 1984; Wyatt, 1988; Kishimoto et al., 1999). Cells isolated from fat body tissue were reported to initially grow slowly, but can be established as continuous lines (see Table 3). For instance, a fat body cell line isolated from *M. brassicae* was reported to be first passaged after 26 days, but subsequently 100 passages were performed over a 9-month period (Mitsuhashi, 1981). Doubling times for fat body cell lines ranged from 48-72 h (Mitsuhashi, 1981, 1983, 1984). Fat body cells can be stored temporarily at 5°C or for years at −70°C in 10% glycerol (Mitsuhashi, 1981).

Table 2. Cell types within insect fat body tissue.

Year	Species	Common name	Cell line	References
1981	*Mamestra brassicae*	Cabbage armyworm	NIAS-MaBr-85	Mitsuhashi, 1981
1982	*Periplaneta americana*	Cockroach	Apa	Philippe, 1982
1983	*Leucania separate*	Common armyworm	NIAS-Le-Se-11	Mitsuhashi, 1983
1984	*Spilarctia seriatopunctata*	Arctiid moth	NIAS-SpSe-1	Mitsuhashi, 1984
1988	*Spilosoma imparilis*	Mulberry Tiger moth	FRI-Splm-1229	Mitsuhashi and Inoue, 1988
1991	*Lymantria dispar*	Gypsy moth	IPL-LdFB	Ferkovich et al., 1991

Table 3. Continuous cell lines established from insect fat body tissue.

Cell Type	Description	References
Trophocyte	Also known as "fat cells", differentiated from mesodermal precursors, store energy	Baryshyan et al., 2012
Vitellphage	Also known as "yolk cells", work to breakdown embryonic yolk components and contain lipids, proteins and carbohydrates	Baryshyan et al., 2012; Hoshizaki and Chapman, 2012
Oenocyte	Present in Diptera and Coleoptera, might metabolize ecdysteroids	Raikhel et al., 1997
Mycetocyte	Present in Hemiptera and Orthoptera	Raikhel et al., 1997
Urocyte	Contain large crystals of uric acid, store nitrogen metabolites as urates	Raikhel et al., 1997; Hoshizaki and Chapman, 2012

Media Formulation

A key benefit of insect cells compared to mammalian cells is ease of adaptation to serum-free media. The use of animal-derived serum in culture media for cellular agriculture is problematic due to high costs, batch-to-batch variability, and ethical concerns related to animal welfare. Fortunately, insect cells are frequently adaptable to serum-free media, and a wide variety of suitable formulations are commercially available or reproducible from published reports (Chan and Reid, 2016). Scale-up of insect cell culture for industrial applications has fueled successful efforts to develop serum-free media at a low price point, indicating promise for economic viability of insect cell-based food production (Donaldson and Shuler, 1998). However, further innovation in this area is needed, as most media options continue to rely on chemically undefined components that limit precise optimization and analysis (Agathos et al., 1990; Mitsuhashi and Goodwin, 2018). Some early studies detail "in-house" serum-free media formulations for insect cell cultures. For example, Donaldson and Shuler (1998) developed a low-cost serum-free media formulation (IPL-41 basal medium, soy protein hydrolysate, yeastolate, lipid-sterol emulsion, and Pluronic F-68) that supported growth rates of the High Five cell line equivalent to the commercial media formulation, Ex-Cell 405.

Scaffold Biomaterials

To date, three-dimensional insect tissue constructs have primarily been developed to generate bioactuators. Primary muscle cultures from *M. sexta* (Baryshyan et al., 2014) or dorsal vessel explants from *C. agnate* (Akiyama et al., 2012a, 2013) have been integrated with polydimethylsiloxane (PDMS) molds to generate mechanical systems. PDMS is used as a morphological guide to encourage self-assembly of primary cultures and to provide a flexible substrate during muscle contractions. Scaffolds for cultured meat require disparate design requirements. For example, a scaffold intended for ingestion should be free from animal components, inexpensive, abundant, and edible. In addition, similar to scaffolds for biomedical applications, the structure should have a large surface area for cell adhesion and mimic the mechanical properties of target tissues. To develop cultured insect tissues for human consumption, scaffolding materials will need to be explored, characterized, and optimized with these features in mind.

Mushroom chitosan may be a promising biomaterial for insect tissue scaffolds. It meets the above design requirements and chitosan is well-documented in the literature for scaffold fabrication in the biomedical engineering field (Croisier and Jérôme, 2013). Specifically, chitosan can be constructed into sponges with aligned pores that mimic the extracellular matrix of skeletal muscle tissue (Jana et al., 2013). Chitosan may be specifically relevant to insect and crustacean tissue engineering as the material is derived from chitin, a principle component of invertebrate exoskeleton (it can also be isolated from fungi) (Pochanavanich and Suntornsuk, 2002; Moussian et al., 2005). In insect cuticle, chitin is present in the form of nanofibril bundles (2–6 nm diameter, 500 nm length) (Muzzarelli, 2011). Although crustacean-derived chitosan is more commonly utilized than mushroom-derived chitosan, fungal chitosan could present additional benefits (besides being animal-free) including more specific molecular weight and absence of allergens (Croisier and Jérôme, 2013). Furthermore, mushroom chitosan is "generally recognized as safe" (GRAS) by the United States Food and Drug Administration. Chinova Bioworks produces chitosan as a preservative for the food and beverage industry as the material is proposed to be effective against a range of microorganisms, as well as digestible as a healthy dietary fiber (Daniells, 2018).

Aside from chitosan, other biomaterials commonly used for medical applications of tissue engineering may be suitable for cellular agriculture purposes (see Table 4). However, few materials have been evaluated specifically for insect-based tissue engineering, and it is therefore important to investigate fundamental properties such as the capacity of materials to support insect muscle and fat cell adhesion, survival, proliferation and differentiation.

Biomaterial	Description	References
Alginate	A polysaccharide isolated from algae, commonly used as a hydrogel, can be functionalized with ligands to promote cell adhesion	Rowley et al., 1999
Cellulose	Cellulose-rich plants can be decellularized to create a cellulose scaffolds with native structure to guide cell development or provide conduits for vasculature	Modulevsky et al., 2014; Gershlak et al., 2017
Chitosan	Derived from crustacean and fungal sources, can be constructed as films, sponges or hydrogels, may provide antimicrobial benefit	Croisier and Jérôme, 2013
Silk	Traditionally produced by silkworms or spiders, can be produced via recombinant protein technology, biocompatible, edible and customizable	Rockwood et al., 2011
Starch	Derived from corn, potatoes and sweet potatoes, composed of amylose and amylopectin, can be processed into porous scaffolds or hydrogels	Nakamatsu et al., 2006; Van Vlierberghe et al., 2011

Table 4. Biomaterials potentially suitable for cellular agriculture scaffold applications.

Bioreactor Design

While most cell culture systems require adhesion, many insect cell lines can be readily adapted to suspension culture in spinner flasks or bioreactors (Palomares et al., 2015). The surface-to-volume ratio of stationary monolayer cultures decreases as scale increases, making such systems problematic for mass production. Thus, the capacity to grow in suspension culture is useful for cost-effective, high-volume cell generation (Wu et al., 1989; Agathos, 1991). Protocols have been established for the adaptation of insect cells lines to suspension conditions, and previous efforts have been successful in establishing systems suitable for scalable culture (Murhammer and Goochee, 1988; Gioria et al., 2006). Alongside traditional batch bioreactors, a variety of more complex bioreactor systems have been evaluated for insect cell culture applications for the recombinant protein production industry including: airlift, semi-continuous, rotating wall, and perfusion reactors (Ikonomou et al., 2003). While simple bioreactor schemes could be useful for cell proliferation phases, perfusion reactors could be utilized for the muscle differentiation phase during which the cells would be adhered to a surface as multinucleated myofibers.

Other Considerations

Transfection

Efficient transfection systems have been developed for introducing recombinant DNA into insect cells (Roest et al., 2016; Mori et al., 2017). While this technology has primarily been employed for industrial production of recombinant proteins, it also shows promise for application in the production of insect cells as food (Ikonomou et al., 2003; Drugmand et al., 2012). By transfecting specific genes into insect muscle cells, it will be possible to create insect cell-based food products with optimized nutritional qualities, taste, and texture. Genes could also be introduced to expand proliferative capacity of primary cultures, reduce the dependence of cells on expensive growth factors, or incorporate clinically relevant proteins for development of therapeutic foods.

Relevance to Crustacean Cell Culture

Advances in insect cell culture and tissue engineering can potentially be translated to *in vitro* methods for invertebrate species that are more readily consumed in Western cultures such

as lobster, crab and shrimp due to evolutionary proximity of insects and crustaceans. Although hundreds of insect cell lines have been developed, no continuous crustacean cell lines have been established to date (Claydon, 2009). However, primary crustacean cell culture methods are commonly adapted from methods developed for insect cells. For example, the basal culture media for crustacean cultures typically consists of Grace's Insect Medium (Toullec, 1999; Li and Shields, 2007). One study detailed the relevance of insect methods to the culture of prawn cells, noting knowledge of animal body fluid composition, patience with slow initial culture growth, and low temperature incubation as important factors for success (Lynn, 1999). Pursuing the continued application of insect cell technology for the development of crustacean culture systems may help accelerate the arrival of cultured crustacean food products.

Nutrient Profile

Edible insects are often lauded for having equal or superior nutrient profiles relative to conventional meat or poultry products, as they are a dense source of protein, monounsaturated and polyunsaturated fats, fiber, and minerals (see Figure 1) (Verkerk et al., 2007; van Huis et al., 2013; Latunde-Dada et al., 2016). However, additional research is necessary to determine how the nutrition of cultured tissues compares to values obtained from whole organisms. A 2013 review by the FAO reports that whole insects could provide greater amounts of micronutrients than isolated portions of the insect (van Huis et al., 2013), indicating a heterogenous nutrient distribution. By growing different insect tissues *in vitro*, it will be possible to prioritize the use of nutritious tissues (e.g., muscle, fat) while avoiding those that lack advantageous nutrient profiles. Bioavailability is another relevant concern from a nutritional perspective, as bioavailability of nutrients can vary between organisms and is not always proportional to nutrient concentration. Fortunately, bioavailability of nutrients from cultured cells and tissues can be evaluated via *in vitro* assays with the use of human intestinal cell cultures (e.g., Caco-2) (Maznah, 1999; Latunde-Dada et al., 2016).

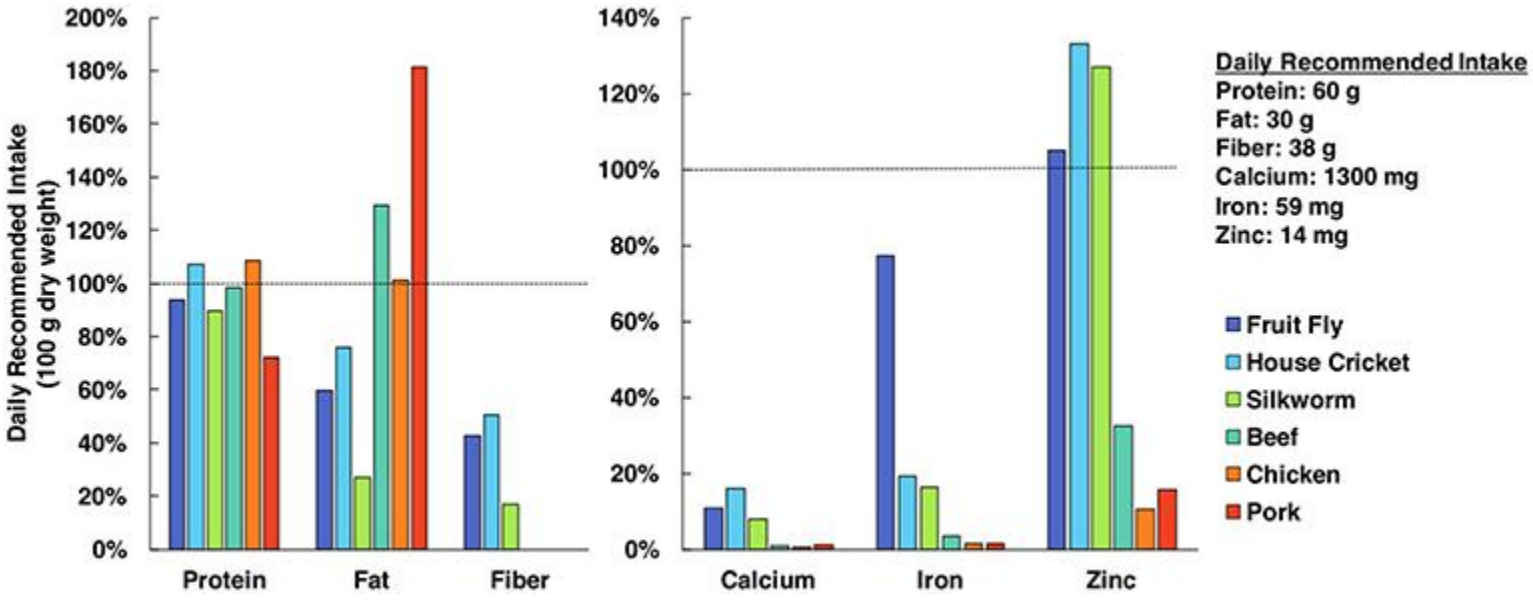


Figure 1. Nutrient profile comparison of edible insects and popular mammalian meats (Rumpold and Schlüter, 2013; Nutrient Data Laboratory, 2018). Nutrients are quantified in

terms of percent of daily recommended intake (DRI) in a 100 g sample (dry basis). Protein, fat and fiber DRI values are specifically for men <50 years old and weighing 75 kg (Institute of Medicine, 2005) and mineral DRI values were selected as the greatest value from the respective ranges (Food Agriculture Organization/World Health Organization, 2004).

Conclusions

The development of a sustainable, healthy, and ethical global food supply is a high priority as the world's population expands. Feeding nine billion people by the year 2050 will require highly efficient food production systems that make responsible use of natural resources and eliminate the harmful effects of high-density animal farming. Cellular agriculture is a rapidly growing field of innovation through which such a food system can be achieved without requiring consumer behavior change. The application of insect cell culture in cellular agriculture shows promise as a means of overcoming technical restraints and achieving high-volume, low-input, and nutritious food production. The robustness of established techniques for culturing insect cells and their ease of immortalization, transfection, high-density proliferation, and serum-free and suspension culture adaptation relative to mammalian cells make them ideal candidates for incorporation into cultured meat and other innovative food products (see Figure 3).

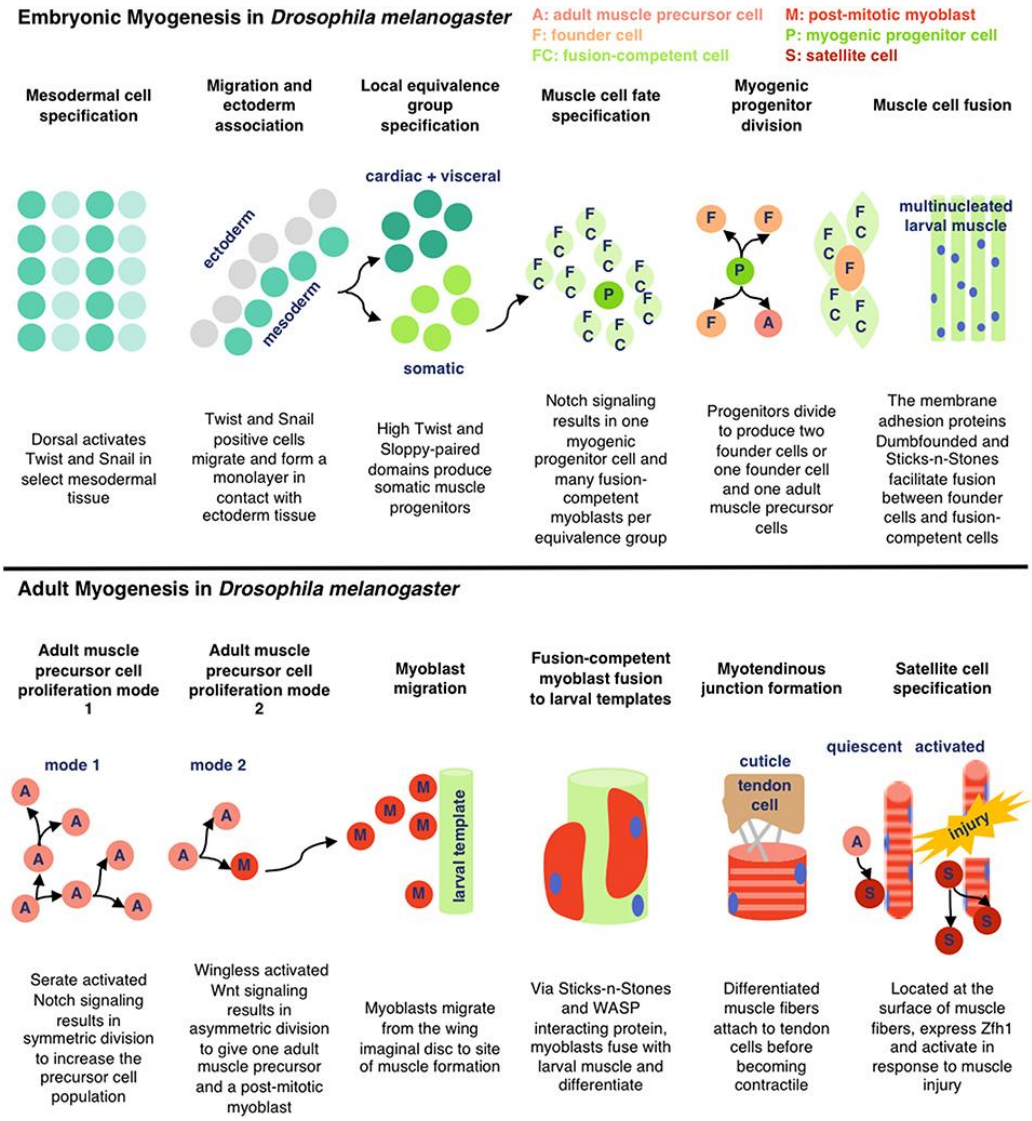


Figure 2. Simplified schematic of embryonic and adult muscle development in *D. melanogaster* as detailed in Gunage et al. (2017) (License: CC BY 4.0). The adult muscle development is specific for dorsal longitudinal indirect flight muscle.

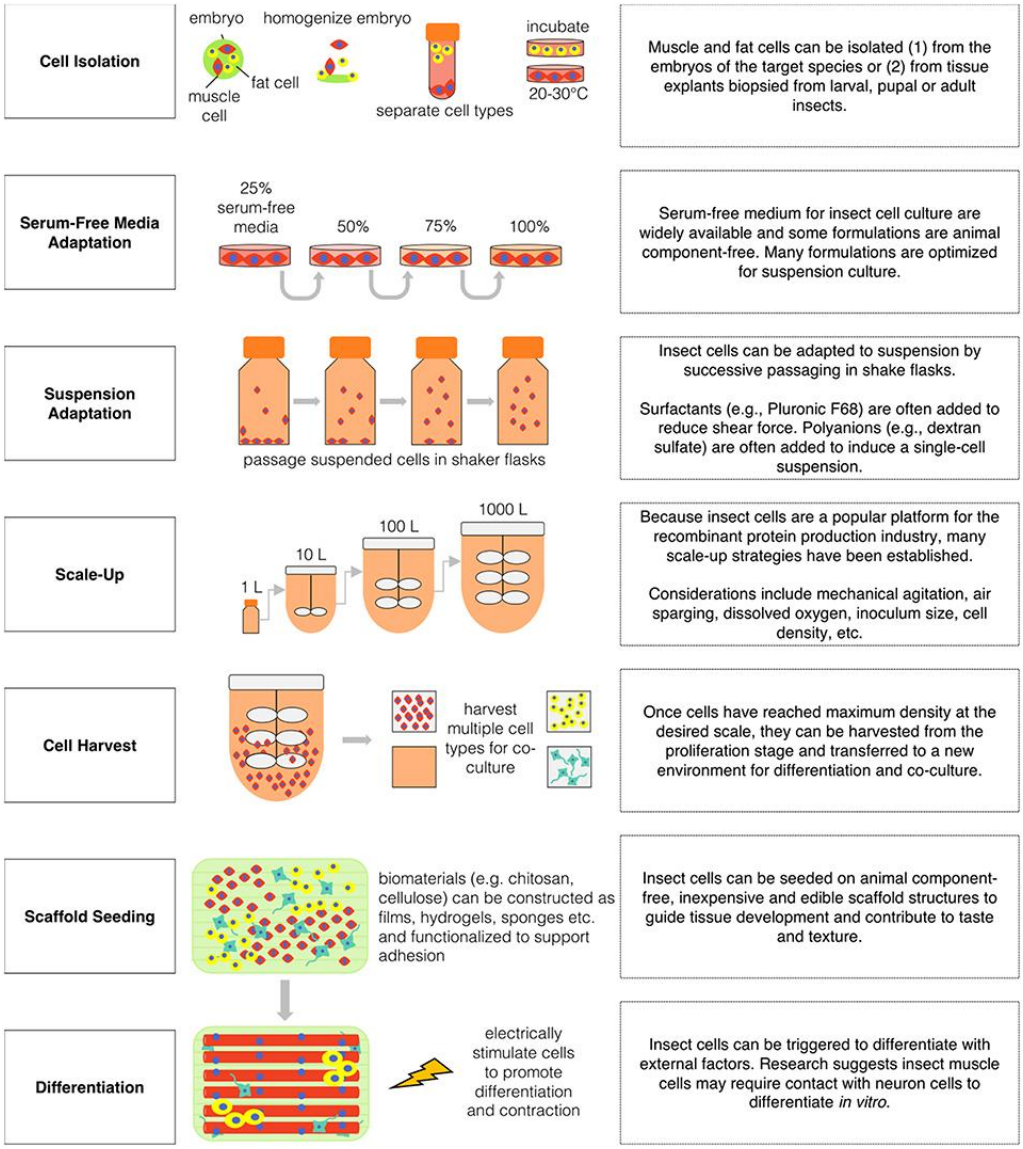


Figure 3. Hypothetical production scenario for insect-based cultured meat.

Although current insect cell culture and biomanufacturing technologies provide a strong foundation for insect cell-based food production, further innovation is necessary to bring this concept to fruition. First, advancements in 3D culture and scaffolding materials are necessary to allow for incorporation of insect cells into large, complex tissues and to mimic the textures of traditional meat and other food products. Second, effective controls of insect muscle cell differentiation are needed. Production at scale will require proliferating high densities of cells and subsequently triggering differentiation into the desired cell types (e.g., muscle, fat) at precise time points. Further research is also needed on the bioavailability of nutrients in relevant cell types, to further elucidate the health impacts of insect cell consumption.

Independent of technology, consumer acceptance may be a barrier to the adoption of insect cell-based foods and other products generated through cellular agriculture. Wilks and Phillips (2017) surveyed consumer attitudes toward the possibility of consuming cultured meat and found

that although most respondents were open to trying the products, few showed a willingness to adopt them as a replacement for traditional animal-based meat. For foods that incorporate cultured insect cells, the barriers to consumer acceptance may be even greater. Particularly in Western countries, the concept of insect-derived foods is unfamiliar and potentially unappealing to most consumers. It will thus be beneficial in the early stages of this technology to incorporate cultured insect cells into existing foods and attempt to mimic familiar tastes and textures to highlight benefits without sparking aversion. Eventually, the application of insect cell culture may allow for the generation of entirely new food products that expand the frontiers of taste and nutrition. In all cases, successful release and adoption of food products generated through insect cell culture will require a tactful approach to public relations and marketing to ensure consumer comfort and receptivity.

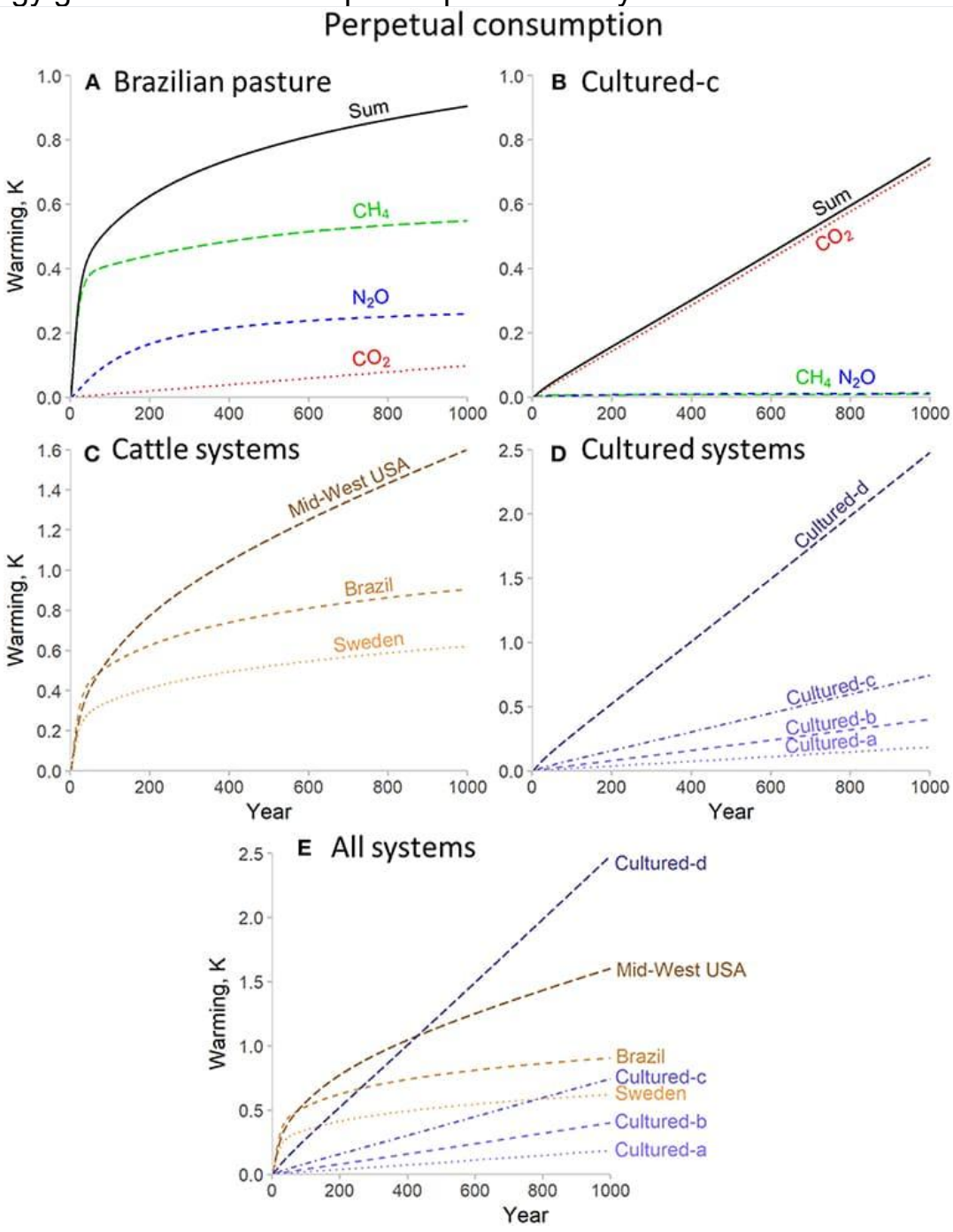
As additional research is conducted on invertebrate cellular agriculture, new findings can be explored for applications to other projects within the cellular agriculture space. For instance, further elucidation of the mechanisms through which insect cells retain function in variable environmental conditions could be translated to improve bovine and porcine cells for large-scale culture. A clearer understanding of the adaptability of insect cells to serum-free media and suspension culture could similarly be applied to advance technology for other cell types. Actively pursuing these connections between disciplines will not only benefit specific product development efforts, it will also strengthen the field of cellular agriculture as a whole.

Climate Impacts of Cultured Meat and Beef Cattle

John Lynch, and Raymond Pierrehumbert

Improved greenhouse gas (GHG) emission efficiency of production has been proposed as one of the biggest potential advantages of cultured meat over conventional livestock production systems. Comparisons with beef are typically highlighted, as it is a highly emissions-intensive food product. In this study, we present a more rigorous comparison of the potential climate impacts of cultured meat and cattle production than has previously been made. Warming impacts are evaluated using a simple climate model that simulates the different behaviors of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), rather than relying on carbon dioxide equivalent (CO_2e) metrics. We compare the temperature impact of beef cattle and cultured meat production at all times to 1,000 years in the future, using four synthetic meat GHG footprints currently available in the literature and three different beef production systems studied in an earlier climate modeling paper. Cattle systems are associated with the production of all three GHGs above, including significant emissions of CH_4, while cultured meat emissions are almost entirely CO_2 from energy generation. Under continuous high global consumption, cultured meat results in less warming than cattle initially, but this gap narrows in the long term, and in some cases, cattle production causes far less warming, as CH_4 emissions do not accumulate, unlike CO_2. We then model a decline in meat consumption to more sustainable levels following high

consumption, and show that although cattle systems generally result in greater peak warming than cultured meat, the warming effect declines and stabilizes under the new emission rates of cattle systems, while the CO_2 based warming from cultured meat persists and accumulates even under reduced consumption, again overtaking cattle production in some scenarios. We conclude that cultured meat is not *prima facie* climatically superior to cattle; its relative impact instead depends on the availability of decarbonized energy generation and the specific production systems that are realized.



Cellular Agriculture: Techniques and Applications

A revolution in the production of food and materials.

Published: August 4, 2025 Written by Rui Rodrigues, PhD

Cellular agriculture is revolutionizing the production of food and materials by using cells instead of whole organisms, offering more sustainable, safe and ethical alternatives. This article explores the techniques, applications and potential of cellular agriculture, focusing on its impact on food production and material innovation.

What is cellular agriculture?

Cellular agriculture is an emerging field that focuses on the production of agricultural products directly from cell cultures instead of relying on whole animals or plants. These products can be divided into two categories: **cellular** products (like cultured meat, leather, or seafood, produced from actual animal and plant cells) and **acellular** products (like milk proteins or egg whites, produced from cultures of microorganisms – e.g., bacteria, yeasts, fungi, and algae – via fermentation).[1]

Cellular agriculture combines several scientific fields, including **biotechnology, molecular biology, tissue engineering, and synthetic biology**, to create sustainable alternatives to conventional farming. It aims to produce agricultural products that closely replicate the molecular, sensory, nutritional, and structural qualities of those made through conventional methods. The benefits include reduced environmental impact, less land and water use, fewer greenhouse gas emissions, and ethical advantages by eliminating large-scale animal farming and reducing disease risks.[3] It also provides greater food security with controlled production and allows precise control over nutritional content.[4]

The concept dates back nearly a century. In 1932, Winston Churchill famously predicted that humans would one day grow only the edible parts of animals, envisioning a future where meat could be produced without raising whole animals. However, it wasn't until the 21st century that this concept became real, driven by accumulated scientific knowledge and technological innovations. In 2013, the world saw the first lab-cultured hamburger, grown from cow muscle cells by Dr. Mark Post and his team at Maastricht University.[5] Since then, cellular agriculture has transformed from a scientific curiosity to a rapidly developing field, attracting billions in investment and leading to the creation of many startups, research labs, and pilot manufacturing facilities around the globe.[6,7] An overview of the general cellular agriculture process for cultured meat production is presented in Figure 1.

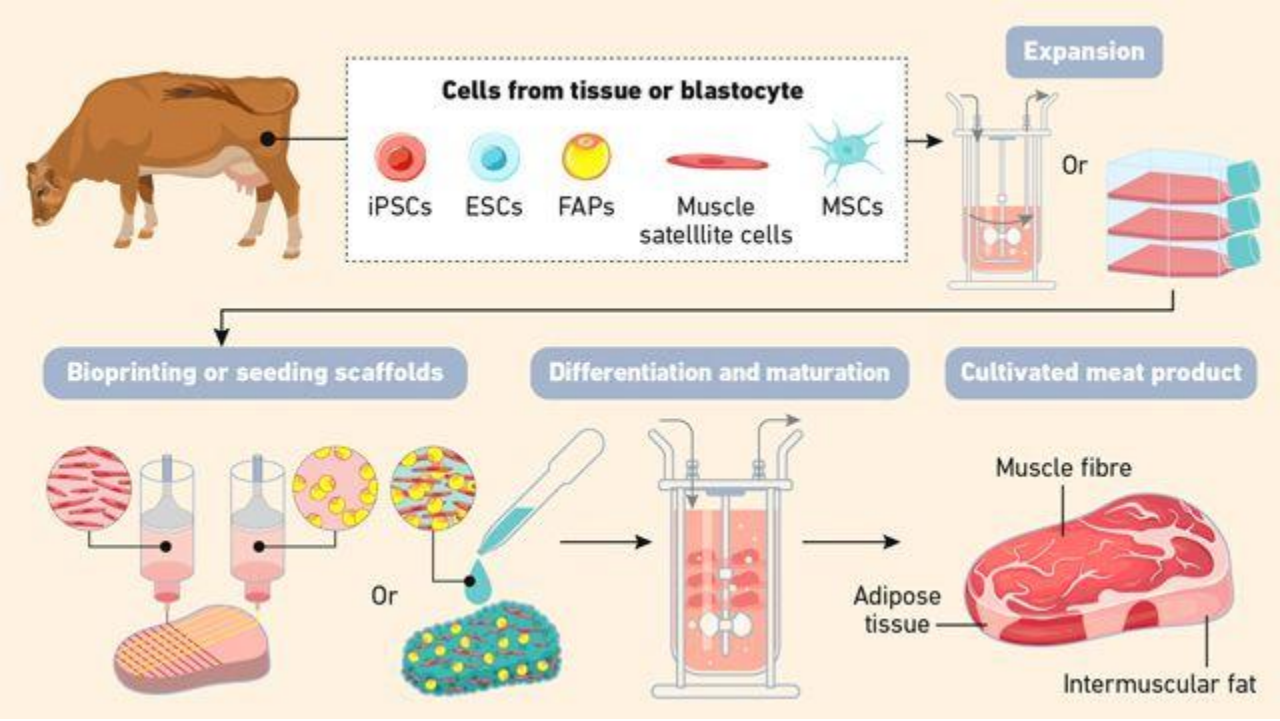


Figure 1: Overview of the general cellular agriculture process for cultured meat production. *Credit: Technology Networks.*

How does cellular agriculture work? Tissue engineering meets synthetic biology

At the core of cellular agriculture are two main approaches:

1. **Cell culture-based production**: This uses tissue engineering to grow animal or plant cells directly into structured products, such as meat or leather.
2. **Fermentation-based production**: This uses naturally occurring or genetically engineered microbes to produce specific proteins, fats, and other biomolecules.

Culturing animal and plant cells – including cultured meats

Culturing animal and plant cells involves isolating specific cell types – such as **muscle satellite cells or plant parenchyma cells** – and growing them ***in vitro*** under controlled conditions, in bioreactors.[8] Plant cells can be used in cellular agriculture, primarily for producing rare plant-based ingredients (e.g., saffron, vanilla, or ginseng), pigments, or biopharmaceuticals. However, it plays a minor role compared to animal cell culture or precision fermentation due to limited efficiency and scalability.

The process of creating cultured meat or other tissue-based products typically begins with the selection and cultivation of specific cell types (Figure 1). In the case of meat, satellite cells – a type of muscle stem cell – are isolated from a living animal. These cells are chosen for their ability to **proliferate** and **differentiate** into mature tissue types like muscle and fat. Once isolated, the cells are placed in a **growth medium** that provides the necessary nutrients and growth factors to support **cell proliferation**. As the cells multiply, they are transferred onto **scaffolds** – three-dimensional (3D) structures that resemble natural tissues – into the **bioreactor.** Scaffolds are biocompatible frameworks made from natural or synthetic materials (e.g., collagen, alginate, cellulose). They not only provide a physical surface for cells to adhere to and grow upon, but also help replicate the **texture, mouthfeel, and appearance** of natural cellular tissue.[9] Advanced methods like 3D bioprinting may be used to layer cells in specific patterns, mimicking the texture and complexity of animal meat.[1]

Cultured meat is starting to be approved in a few countries, sustained by scientific, regulatory, ethical, and economic factors. Singapore, the U.S., and Israel have allowed cell-based meat, such as cultivated chicken, and the UK has approved it for pet food.[8]

Culturing microorganisms – precision fermentation

Culturing microorganisms is a foundational method in cellular agriculture, particularly for producing animal-free food ingredients, materials, and functional compounds. This approach leverages microbes (like yeast, bacteria, or fungi) to use the whole biomass or produce target compounds. Fungal culture is a powerful method in cellular agriculture and alternative protein

production, where filamentous fungi are cultivated to produce high-protein food ingredients. This process involves growing fungi in controlled fermentation tanks, feeding them with nutrients, and harvesting the biomass for use in food products. Quorn® is one of the most well-known and widely consumed products made from fungal mycoprotein. Another established line is microalgal cultivation to produce food, feed, or high-value compounds without relying on traditional farming. Microalgae can be used as food supplements or for the production of protein-rich ingredients for alternative meat and dairy. Organisms such as spirulina and chlorella are used for natural food coloring, and some microalgal strains can also produce omega-3 oils used as fish oil replacements. Microalgal cultivation is a growing branch of cellular agriculture, especially with respect to producing alternative proteins, lipids, or functional food ingredients through cell-based cultivation.[10]

Precision fermentation is an emerging biotechnology process used to produce specific functional ingredients by programming microorganisms (Figure 2). It involves the insertion of specific genes to **modify microorganisms**, such as yeast, bacteria, or fungi, to produce specific biomolecules, including **proteins, fats, pigments, and enzymes**, which can improve the quality, flavor, safety, and sustainability of foods.[9] By harnessing this technology, food producers can create more consistent and natural ingredients while reducing reliance on animal-based products and minimizing environmental impacts. It offers a promising solution for enhancing food production, making it more efficient and eco-friendly, while also ensuring safer and more nutritious products.[11]

The process starts by inserting a gene encoding the desired protein into the genome of a microorganism. These engineered microbes are then cultivated in bioreactors – **fermentation tanks** – where they metabolize sugars and other nutrients and produce the target biomolecules. After fermentation, the protein is extracted, purified, and processed into a variety of products.[12]

Unlike cell-cultured tissue products, fermentation-based outputs are typically **acellular** – they don't involve the cultivation of animal cells but instead focus on replicating the biochemical components of animal products. Currently, there are a number of companies producing animal-free dairy proteins, such as whey, casein, and egg white protein, and even collagen for leather.[12]

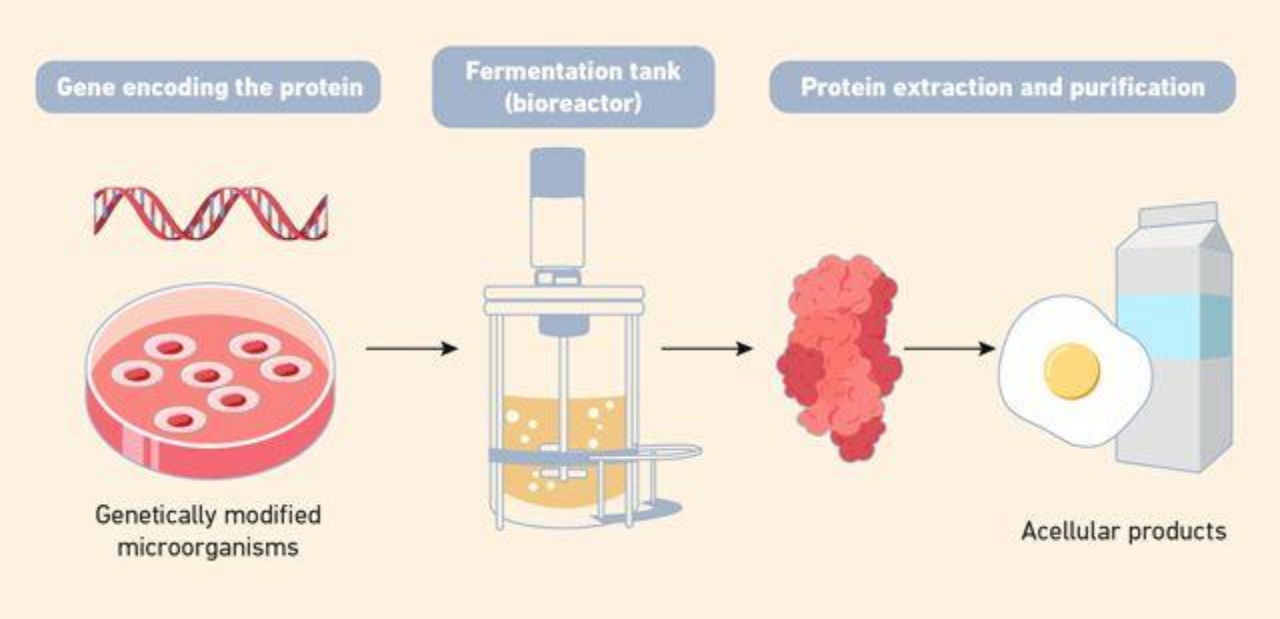


Figure 2: Schematic representation of the production of acellular products via precision fermentation. (Microorganisms are genetically engineered to produce specific proteins and compounds, which are then harvested without using animal cells.) *Credit: Technology Networks.*

Growth media considerations

Growth media is essential in cellular agriculture, as it provides cells with nutrients such as **amino acids, glucose, salts, and vitamins**. These compounds support the growth, division, and specialization of the cells. In traditional cell culture, **fetal bovine serum (FBS)** was often used due to its rich composition of growth factors and nutrients essential for cell proliferation. However, concerns over ethical issues, batch variability, contamination risks, and high costs have driven the search for alternatives.[13] Cellular agriculture aims to use **serum-free, animal-free, antibiotic-free, and plant-based media** that are more sustainable and scalable. These new media include plant hydrolysates, recombinant proteins (produced via precision fermentation), synthetic media formulations, and optimized nutrient blends tailored to specific cell types.[14] In addition to being more in line with environmental and animal welfare goals, these improvements significantly reduce costs and increase the scalability for large-scale manufacturing. A comparison of traditional and cellular agriculture media is shown in Table 1.

Table 1: A comparison of growth media used in traditional cell culture and cellular agriculture.

	Traditional Media (FBS)	Cellular Agriculture Media
Source	Animal-derived (fetal bovine serum)	Plant-based, synthetic or recombinant
Composition	Naturally rich in growth factors and nutrients	Customizable, defined blends (plant hydrolysates, proteins)
Ethical Concerns	High – involves animal slaughter	Low – animal-free, cruelty-free
Batch Consistency	Variable between batches	More consistent and controlled
Contamination Risk	Higher risk of pathogens and impurities	Lower – sterile and defined components
Cost	High and increasing	Potentially lower with scale
Scalability	Limited by the supply of FBS	Designed for large-scale, sustainable production
Suitability for Cell Types	Broad, but not tailored	Can be optimized for specific cell types

Bioreactors for scaling up production

To meet market demand, cellular agriculture must scale up from laboratory- to industrial-scale production using **bioreactors**. These are controlled vessels that maintain **optimal conditions** – such as temperature, pH, oxygen, and nutrient levels – to support consistent and efficient cell growth. Bioreactors allow cells to be mixed, agitated, and aerated to ensure proper distribution of nutrients and gases throughout the culture.[1]

The production process of cellular agriculture typically begins with **cell expansion**, which involves a sequence of bioreactors of increasing volume. This setup helps maintain exponential cell proliferation while preventing premature differentiation. During this stage, **stirred-tank bioreactors** are commonly used for culturing animal cells in suspension. They offer effective mixing, oxygen transfer, and foam management, all of which are crucial for supporting both the growth and eventual differentiation of stem or progenitor cells. Once a sufficient number of cells have been cultivated, they are attached to **scaffolds** or arranged into 3D structures through **3D bioprinting**. These structures are then transferred into **perfusion bioreactors**, which are specifically designed for the **maturation phase**. Here, the cells develop into functional tissue – such as muscle or fat – under precisely controlled flow conditions that support nutrient delivery,

waste removal, and tissue structuring.[15] A representative diagram of the whole process is presented in Figure 3.

In **precision fermentation**, a similar stirred-tank bioreactor setup is used, often incorporating **microcarriers**—tiny spherical particles that increase the available surface area for cell growth. This approach focuses on optimizing microbial productivity, allowing engineered microorganisms to produce target proteins, enzymes, or fats efficiently, while also simplifying the process of downstream purification and extraction.[16]

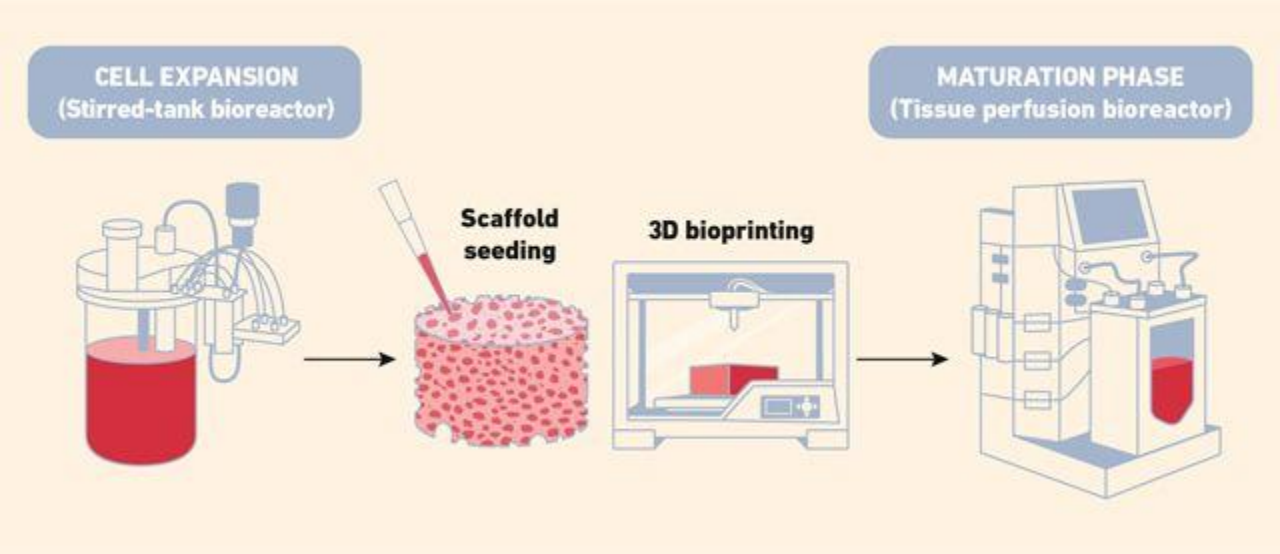


Figure 3: Bioreactors used for scaling up production in cellular culture. (These systems support controlled cell growth and tissue development under optimized conditions for large-scale manufacturing.) *Credit: Technology Networks.*

The role of 3D bioprinting in cellular agriculture and 3D printed food

In cellular agriculture, 3D bioprinting is used to manufacture structured and realistic food products, such as cultured meat, through the **layering of cells and biomaterials in precise, programmable patterns**. By using this method, it is possible to mimic the composition, texture, and appearance of typical meat cuts, including the appearance of **fat marbling and muscle fibers.**[17]

A crucial element in this process is the **scaffold**, which offers a 3D structure that facilitates tissue development, cell adhesion, and growth. Scaffolds can appear in the form of hydrogels, fiber scaffolds, and microcarriers. These can be produced via 3D printing or extrusion methods, which are further subdivided into fused deposition modeling (FDM) and extrusion modeling (EM). Scaffolds are made from a range of materials, such as synthetic or bioengineered materials (like polylactic acid (PLA) or alginate), animal-derived proteins (like collagen or gelatin), and edible

plant-based polymers (like cellulose or soy protein). Each variety has unique mechanical strength, porosity, biodegradability, and biocompatibility characteristics. Furthermore, the ideal scaffold should facilitate the flow of nutrients and oxygen, promote cell development, and be safe for consumption.[18]

In addition to creating structured meats like steaks, burgers, and fillets, bioprinting also facilitates **personalized nutrition**, adapting food products to meet specific consumer preferences and needs.[19]

Pros and cons of cellular agriculture

Cellular agriculture has great potential to produce animal-based products and alternatives in a sustainable and ethical way. But despite its many advantages, several challenges remain unsolved. Weighing the pros and cons is essential to ensure responsible development, public trust, and comprehensive food system improvement. Understanding both sides helps to guide smart policy, investment and research decisions for a more ethical and resilient food future.

Pros

- **Environmental sustainability:** Reduces greenhouse gas emissions, water usage, and land use.
- **Animal welfare:** Eliminates the need for raising and slaughtering animals.
- **Food security:** Offers a stable food supply that is less vulnerable to climate change or pandemics.
- **Customization:** Enables tailored nutrition and functionality.
- **Scalability:** Potential for mass production with fewer natural resources.

Cons

- **High costs:** Still relatively expensive compared to conventional products.
- **Regulatory hurdles:** Unclear regulations in many regions.
- **Consumer acceptance:** Skepticism about safety and naturalness.
- **Technical challenges:** Difficulty replicating complex tissue structures/textures and flavors.
- **Economic and social changes:** Redefinition of farming and farmers' way of life.

Applications of cellular agriculture, current state, and future perspectives

Cellular agriculture spans from traditional uses in health and alternative foods to cutting-edge applications in animal-free products such as meat and leather (Figure 4). Animal cells and microorganisms can be cultivated in controlled environments, offering a clean, efficient alternative to conventional farming and animal agriculture. These practices are central to building a sustainable, ethical, and innovative food system. Whether through more established practices like fermenting algae and fungi, or modern advances like precision fermentation, they offer scalable solutions to reduce our dependence on animals, lower environmental impact, and meet growing global food demand.

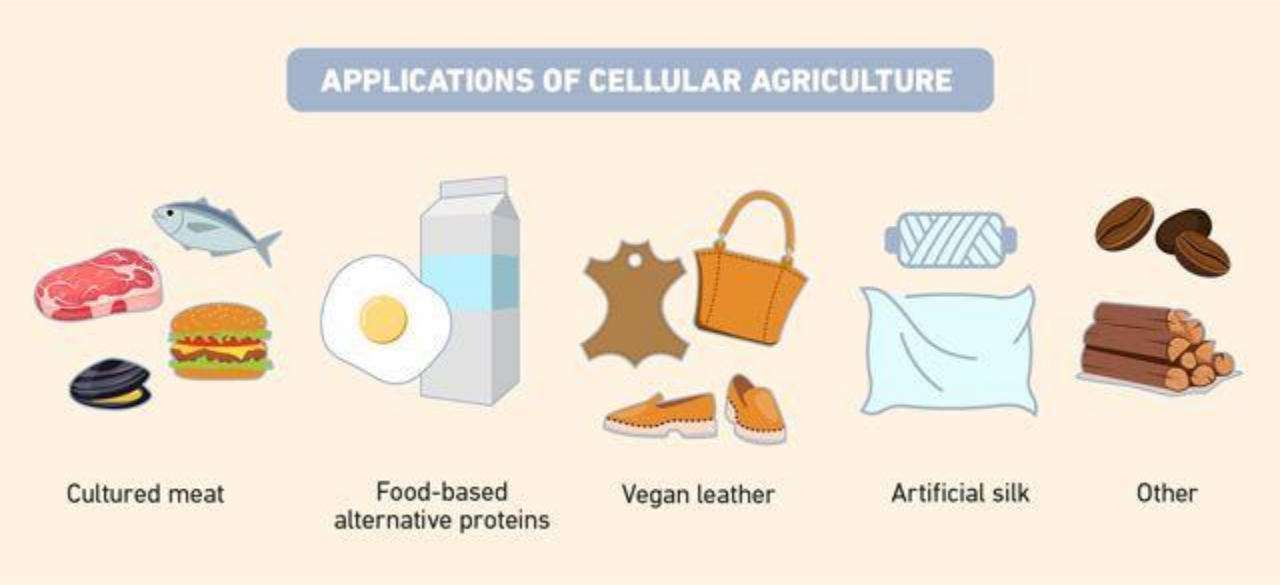


Figure 4: Examples of applications of cellular agriculture. *Credit: Technology Networks.*

Lab-grown meat

Cellular agriculture uses tissue engineering to grow animal muscle cells in bioreactors, producing cultured meat products such as beef, poultry, pork, and seafood in the form of burgers, nuggets, and fish fillets that closely mimic conventional flesh in taste, texture, and nutritional value.[20,21] These products have been produced at pilot and pre-commercial scales and are valued for their potential to reduce environmental impact and animal suffering [1,3]

Other food-based alternative proteins

Through precision fermentation, microorganisms are engineered to produce animal proteins like casein (milk protein), ovalbumen (egg white protein), and whey protein, which are then purified and used in foods such as animal-free dairy products and egg substitutes. These proteins are already incorporated into commercially available products and offer vegan, allergen-reduced, and sustainable alternatives [1,12]

Vegan leather

Cellular agriculture enables the production of animal-free leather by cultivating structural proteins like collagen or using biological materials such as fungal mycelium and bacterial cellulose. These materials are processed into leather-like sheets used in shoes, bags and textiles, offering a sustainable alternative to traditional leather. Fungal bioleather, especially from *Ganoderma lucidum*, stands out for its biodegradability and low-cost production using agricultural waste. The production of lab-grown collagen-based leather and bacterial cellulose leather is still being studied but shows promise.[22,23]

Artificial silk

Cellular agriculture enables the production of artificial silk by engineering microorganisms and transgenic organisms to produce spider silk proteins, offering a cruelty-free and biodegradable alternative to traditional silk. While natural silk, especially from spiders, is difficult to harvest at scale, recombinant production using hosts like bacteria, yeasts, mammalian cells, and genetically modified silkworms allows silk fibers to be spun that replicate the strength and flexibility of natural spider silk. These fibers are already being used in textiles and high-performance materials across fashion, medical, and industrial applications.[23,24]

Other applications

Plant cell cultures are being developed to produce wood-like materials by cultivating cambial stem cells from trees in a gel medium that encourages them to grow into rigid structures with similar properties to natural wood.[25] Similarly, cultured coffee[26] is produced through microbial fermentation, where specific plant cells or microbes are programmed to biosynthesize coffee compounds like caffeine and aromatic molecules.

In conclusion, cellular agriculture represents a transformative shift in how we produce food,

materials, and other biological products. Leveraging cell culture, tissue engineering, and precision fermentation, it enables the creation of sustainable, ethical, and innovative alternatives to traditional animal and plant agriculture. From cultured meat and dairy proteins to bioleather and functional ingredients made by microalgae, fungi, and yeast, the field is already demonstrating its potential to reduce environmental impact, improve food security, and meet the growing global demand for ethical and efficient food systems.

However, despite its promise, cellular agriculture still faces technical, economic, and regulatory challenges. Scaling up production, reducing costs, and gaining consumer acceptance will be critical for its mainstream success. Continued investment in research, transparent communication, and policy development will be essential to realize the full benefits of this revolutionary field.

Exploring a Just Transition for Livestock Agriculture and Cellular Agriculture

As global food systems adapt to evolving economic and technological realities, livestock agriculture is at a turning point. Changing consumer preferences, regulatory shifts, and new food production technologies are reshaping the sector. To ensure stability and long-term resilience, it is important to develop pathways for adaptation that support farmers, ranchers, and rural communities.

To address this need, Aleph Farms and Federation University Australia have partnered on a research initiative to explore a just transition in livestock agriculture—one that ensures producers can navigate change while maintaining economic security and social inclusion.

The question we need to ask is what a just transition for the livestock industry looks like. While industries are changing due to climate action and regulation, they need new strategies that allow people to adapt without being left behind. But a just transition isn't solely about industries, it's about guiding entire economies toward more sustainable and equitable models. The concept originated in the energy sector, where policies were developed to support workers as industries shifted from fossil fuels to renewable energy. These policies have prioritized economic stability, job creation, and skills development, ensuring that communities historically reliant on traditional sectors are active participants in shaping the future.

While energy transition frameworks are well-established, livestock agriculture lacks a structured approach for managing change. Unlike energy production, which is centralized, livestock farming is deeply embedded in local economies, food cultures, and regional supply chains, employing over 1.3 billion people worldwide.

A just transition for livestock agriculture is not about replacing one system with another. Instead, it is about ensuring that while we transition to more resilient agrifood systems, producers have access to new opportunities while preserving the knowledge, traditions, and livelihoods that define their role in the food system. Diversification, rather than replacement, is key—integrating cellular agriculture, precision fermentation, and regenerative livestock practices in ways that offer producers new tools and markets without disrupting their livelihood.

Led by PhD candidate Priyambada Joshi and Dr. Lee Recht, former VP of Sustainability and Policy at Aleph Farms, this research examines key factors shaping beef production across the United States, France, and soon, Brazil. Through interviews and surveys with over 150 beef producers, the study explores how livestock agriculture and food innovation can complement each other to strengthen the broader food system.

Findings highlight the importance of region-specific approaches in shaping transition strategies:

In the U.S., producers emphasize the need for financial security, land access, and labor stability to ensure long-term resilience. Many are adopting new technologies and diversification strategies to enhance stability in an evolving market.

In France, grass-fed beef producers are adapting to market fluctuations, generational shifts, and structural changes in production systems. Collaborative models, such as shared infrastructure and cooperative investments, are helping support the sector.

And in Brazil, the next phase of the study will offer insights into livestock production dynamics in another major beef-producing country, providing a broader understanding of transition pathways worldwide.

The study shows that livestock farmers face a range of challenges, including production risks (land shortages and climate hazards), financial risks (rising costs of land, labor, and feed), human risks (labor shortages and generational transitions), policy risks (regulatory changes and environmental mandates), and market risks (price volatility in both sales and input procurement).

To manage these uncertainties, many are adopting precision agriculture, sustainable grazing methods, diversified revenue streams, cooperative farming models, and innovative financial tools to enhance resilience.

Over a quarter (27 percent) of livestock farmers surveyed believe that cultivated beef and conventional beef can successfully coexist in the market, while a substantial 42 percent remain open to the conversation. Likewise, around 15 percent see potential for collaboration between beef producers and the cultivated beef industry, with nearly 49 percent still exploring their stance. This high level of uncertainty highlights an opportunity to engage in meaningful discussions on innovation and how cultivated beef can complement sustainable methods of conventional beef production.

Unlike industries where success is measured by replacing outdated systems, food production requires a balance between existing and emerging practices. The transition in livestock agriculture must be inclusive of farmers and ranchers, ensuring they have access to financial resources, technical knowledge, and new market opportunities.

The research highlights how diverse food production methods can work together. Regenerative livestock farming, cellular agriculture, and precision fermentation each play a role in strengthening the food supply, and their integration can create a more flexible, resilient system that benefits all stakeholders.

By fostering knowledge-sharing and collaboration, a just transition will enable livestock producers, food innovators, and policymakers to identify solutions that create value and provide a holistic approach with long-term security for those working in the sector.

A just transition in livestock agriculture requires a diverse range of financial models, policy support, and expanded research to ensure that producers have the tools they need to succeed. To facilitate this transition, policymakers and research institutions should focus on expanding funding for joint research that integrates traditional livestock practices and food innovations; improving rural infrastructure and access to financing to enable diversified production; and developing policy frameworks that support producers through economic and structural changes.

As the food sector evolves, collaboration within the animal agriculture industry will be key. A just transition ensures every stakeholder is an active participant in shaping the future of food, rather than being sidelined by change.

The research provides a foundation for this shift, demonstrating that innovation and tradition can work together to create a food system that values economic stability, social inclusion, and environmental resilience.

By integrating diverse approaches, we can ensure that livestock farmers and ranchers remain central to the food system, creating new opportunities while preserving their essential role in feeding communities worldwide.

What Is Cellular Agriculture and How Does It Work? June 29, 2025

Cellular agriculture is an emerging field of biotechnology that produces agricultural products like meat, dairy, and leather from cell cultures instead of whole organisms. Using techniques from synthetic biology and tissue engineering, this approach cultivates cells in a controlled laboratory setting. This process offers an alternative to traditional farming, moving production from farms to facilities that resemble breweries.

The Scientific Process of Cellular Agriculture

The production process begins by sourcing initial cells from a living animal through a small, minimally invasive biopsy. These starter cells, often stem cells, are selected for their ability to replicate extensively. The goal is to establish a cell line that can grow indefinitely, removing the need to return to the animal for more samples.

Once a stable cell line is established, the proliferation phase begins. The cells are placed in a bioreactor, a large vessel that provides a controlled environment for growth. Inside the bioreactor, the cells are submerged in a nutrient-rich liquid called a growth medium that fuels cell division and multiplication. This solution contains all the necessary components for cell development, including:

- Proteins
- Sugars
- Fats
- Vitamins

For products requiring structure, like a piece of meat, scaffolding and differentiation are used. Scaffolding, often made from plant-based materials, provides a structure for cells to attach to and organize into a three-dimensional tissue. The cells are then encouraged to differentiate into specific types, such as muscle and fat cells, which are the primary components of meat. This process helps the final product develop a texture that mimics conventionally produced meat.

Types of Cellular Agriculture Products

Cellular agriculture yields two distinct categories of products: cellular and acellular. Cellular products are composed of cultured cells grown to form tissues, such as cultured meat. For these products, muscle and fat cells are grown to create beef, chicken, or seafood that is biologically identical to meat from animals, and the method can also produce materials like leather.

Acellular products are not made of cells but are created by them through a process called precision fermentation. This method uses

microorganisms like genetically engineered yeast or bacteria, which are programmed to produce specific organic molecules like proteins and fats. For instance, yeast can produce milk proteins like casein and whey for dairy products, or egg proteins for egg whites. Other examples include producing collagen for gelatin and specific fats for food applications.

Comparison to Conventional Farming

Cellular agriculture uses resources differently from conventional animal farming. Life-cycle analyses suggest that producing meat from cells could require significantly less land and water, with some studies projecting reductions of up to 98% and 90% respectively. Energy inputs may also be lower because resources are directed only toward growing the desired product rather than sustaining a whole animal.

Cellular agriculture improves animal welfare by eliminating the need to raise and slaughter animals for food. A single cell sample can supply a large amount of meat, which addresses ethical concerns associated with industrial livestock farming. For many consumers, this is a primary reason to support the technology.

The controlled environment of cellular agriculture facilities can improve food safety. Products are grown in sealed bioreactors, reducing the risk of contamination from pathogens like Salmonella or E. coli. This process also removes the potential for exposure to zoonotic diseases and eliminates the need for antibiotics.

Market Availability and Regulation

The commercialization of cellular agriculture is in its early stages, with availability varying by country. Singapore was the first nation to approve the sale of cultured meat, with the United States following. In the U.S., the Food and Drug Administration (FDA) and the Department of Agriculture (USDA) share oversight. The FDA regulates cell collection and growth, while the USDA's Food Safety and Inspection Service (FSIS) handles the final production and labeling of meat products.

Consumer access to cultured meat is limited, often restricted to specific restaurants in countries where it is approved. Acellular products from precision fermentation are more widely available. For example, animal-free whey protein is an ingredient in some commercially sold ice creams and cream cheeses.

The industry faces challenges in scaling production and reducing costs to compete with conventional products. Many companies are working to advance the technology and expand operations. Widespread market availability will depend on technological progress, navigating regulations, and gaining consumer acceptance.

INDUSTRIES IN DEPTH

How to maximize the impact of cellular agriculture for sustainable food production

Cellular agriculture can enable widespread production of animal proteins, particularly cultivated beef.

Didier Toubia
Co-Founder and Chief Executive Officer, Aleph Farms

- **Animal agriculture is an important source of nutrition for billions, but food production has a severe negative impact on the climate and environment.**
- **As global demand for animal proteins continues to rise, new methods of production can help relieve the tension between scale and sustainability.**
- **Cellular agriculture can enable widespread production of animal proteins, with cultivated beef well-positioned to become a sustainable consumer choice.**

Animal agriculture is a crucial source of nutrition for billions of people, but many of its conventional practices have passed their maximum scale. The food production systems that fed 1.6 billion people at the turn of the 20th century are today stretched thin trying to feed 8 billion people and counting.

Food production's impact on soil degradation, deforestation, the depletion of natural resources, and emissions of methane and other greenhouse gases have become more severe over time. Climate impact, in turn, exacerbates strain on the production systems themselves, which rely on climate conditions remaining steady.

As alarming as anything else is human-induced biodiversity loss. The fast pace at which animal and plant species are disappearing has prompted scientists to declare that we are already in the midst of a sixth mass extinction.

Intensive agriculture is the main culprit; food production – with its heavy reliance on intensive animal farming – is the primary driver of biodiversity loss, responsible for the vast majority of cases in which animal and plant species are at risk of extinction.

Production of animal proteins and fats needs to diversify

As global demand for animal proteins and fats continues to climb, introducing complementary methods of production – alongside the transition to more sustainable methods of conventional production – can relieve the growing tension between scale and sustainability.

In the face of climate change, resource scarcity, and a rising human population, cellular agriculture is well-suited to help diversify the production of animal proteins and fats. Just as animal agriculture has replicated animal reproduction and growth under controlled conditions for millennia, cellular agriculture continues this same practice at the level of individual cells – the building blocks of animals (and animal meat).

Instead of domesticating an entire animal by nurturing it in a feedlot, cellular agriculture involves domesticating animal cells by nurturing them in a cultivator – a tank that emulates the inside of an animal's body with significantly enhanced resource efficiency and without methane emissions.

Production takes weeks instead of months or years and happens in closed systems geographically close to consumption, including places where raising large numbers of animals is not feasible.

This enables decentralized, short, and predictable value chains, reducing pressure on current methods of production and lessening animal agriculture's overall susceptibility to supply chain shocks, such as wars, extreme weather events, animal disease outbreaks, and global health crises. This resilience and diversification help maintain supply and stabilize prices, further enhancing food security.

For cellular agriculture to achieve long-term growth and success, its first applications should be well-positioned to maximize both impact and value. In this regard, protein and fat products grown from cow cells fit the bill.

Importance of cultivated beef in tackling emissions

Cattle have the highest environmental footprint across all of animal agriculture. Cultivated beef can mitigate a lot of this impact by enabling fewer and better-managed cows.

For instance, cows are the biggest contributor to livestock-induced methane emissions, which account for 37% of all anthropogenic emissions of this greenhouse gas.

Methane has more than 80 times the warming power of CO_2 over its first 20 years in the atmosphere. Cutting these emissions is the fastest way to slow down and reverse global warming, and fewer cows is the most direct way to achieve this.

In addition, a complementary production system for cattle products is key for countries and communities looking to establish food sovereignty despite difficult climate conditions, lack of suitable land, or insufficient water resources.

Currently, the production of beef is more concentrated than that of any other common animal protein, forcing many countries in Asia and the Middle East to remain heavily reliant on imports from Australia and the Americas. This over-concentration is set to get worse as heat waves intensify and premiums on land and other resources become more pronounced.

Producing cultivated beef in closed systems can take place irrespective of climate or the availability of local arable land. It can happen geographically close to consumption to help alleviate food insecurity, empower local communities, and spur economic growth.

Moreover, when cow cells can be grown directly into high-quality food items in a matter of weeks and with incredible precision, the only resources required for production are those needed to grow just the edible parts of the animal. This stands in stark contrast to conventional production, where the meat that is commercialized amounts to only 30-35% of a given cow.

Requiring less space and fewer resources also means less of a need to infringe on natural ecosystems to acquire land and resources in geographies where cattle farming is common today. Maintaining these ecosystems protects biodiversity, which is essential for human survival in the long term.

Pairing impact with value to drive price parity

In a similar fashion to other innovations like electric vehicles and solar panels, cultivated beef will be expensive at first before economies of scale and longer-term process improvements come into play.

This is true for all cultivated meat: across different species, the cost of production is more or less the same, and initial product costs for consumers will be similarly high. What separates beef is that, compared to other common protein products from land animals, it delivers more value in global markets, commanding higher prices per unit than other species.

As a result, cultivated beef has a shorter path to price parity vis-à-vis conventional beef, increasing its capacity to drive both consumer acceptance and positive margins more quickly.

Beyond impact and value, there's another factor that separates beef from the rest of animal agriculture: current manifestations of regenerative agriculture provide beef with very little room. Regenerative cattle farming, where cows are part of an ecosystem's natural carbon cycle, requires far too much land to be able to meet demand for beef.

For this reason, with cattle products, cellular agriculture has an extra foot in the door. For these products in particular, no other technology – conventional or transformative – has the potential to complement regenerative farming and pair sustainability with scalability.

Other applications of cellular agriculture

With the consumption of high-quality animal products on the rise, developing and implementing systematic strategies for diversified production becomes all the more necessary.

Cellular agriculture is poised to enable the widespread production of animal proteins and fats from cells of numerous land and sea species, all of which will contribute to making food systems more secure, resilient and sustainable.

Cultivated beef is singularly well-positioned to complement sustainable methods of conventional beef production. Its success could pave the way for cultivated meat more broadly – including applications of animal cells from other species – to be a viable and sustainable choice for consumers.

How can we produce enough protein to feed 10 billion people?

Was the world's first lab-grown beef burger a taste of things to come?

Lisa Sweet
Head of Future of Protein, COVID Response, and Food-Health, World Economic Forum

Accessible, affordable, healthy, and sustainable protein is critical to human nutrition and economic development.

Yet academic analysis shows it will be impossible for a global population of 10 billion - a figure expected around 2050 - to consume the amount and type of protein typical of current diets in North America and Europe if we want to achieve the UN Sustainable Development Goals (SDG) and meet the targets set by the 2015 Paris Climate Agreement.

In 2018, the media have increasingly reported on alternative proteins such as plant-based burgers, edible insects, lab-grown meats, and other novel products. From how avoiding meat and dairy is the 'single biggest way' to reduce our environmental impact, to news that a plant-based burger made the menu of a major US fast-food chain in September, to the *New York Times'* recent prediction that both meat protein grown in a lab and plant-based main courses will arrive on restaurant menus this year: news highlighting the environmental impacts associated with producing animal-based protein and the latest food technologies and trends intended to solve these challenges have become more mainstream.

But despite this recent attention, the world has a long path to travel to provide its growing population with the quantity and quality of protein needed, at affordable prices and in a manner that is both sustainable and optimal for health.

So how will we get there? A year of research and dialogue among stakeholders of the World Economic Forum has begun to suggest a four-part roadmap for making this future of protein a possibility.

Highlighting the multiple benefits to society of transforming today's protein systems

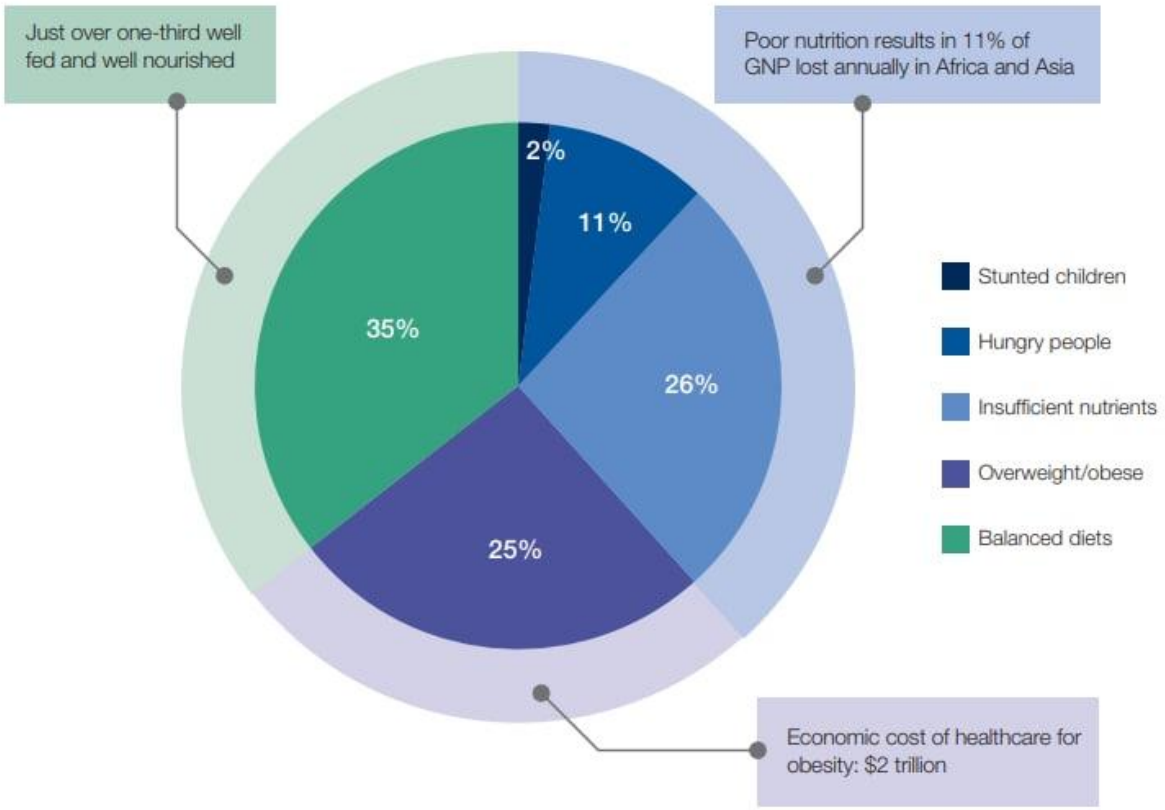
Protein appears quite siloed at first glance, but in reality, it is a topic that sits at the intersection of many agendas, as a few choice statistics demonstrate:

Food: The Food and Agriculture Division of the United Nations predicts that world meat production will double by 2050

Environment: Total emissions from global livestock represent 14.5% of all anthropogenic GHG emissions

Health: Around 65% of the world's population is either undernourished or overweight/obese. While meat is both energy-dense and protein-rich, overconsumption of meat contributes to growing rates of obesity and a higher risk for non-communicable diseases

Livelihoods: Over 1 billion people are involved in livestock value chains, with half of these dependent on livestock for their livelihoods



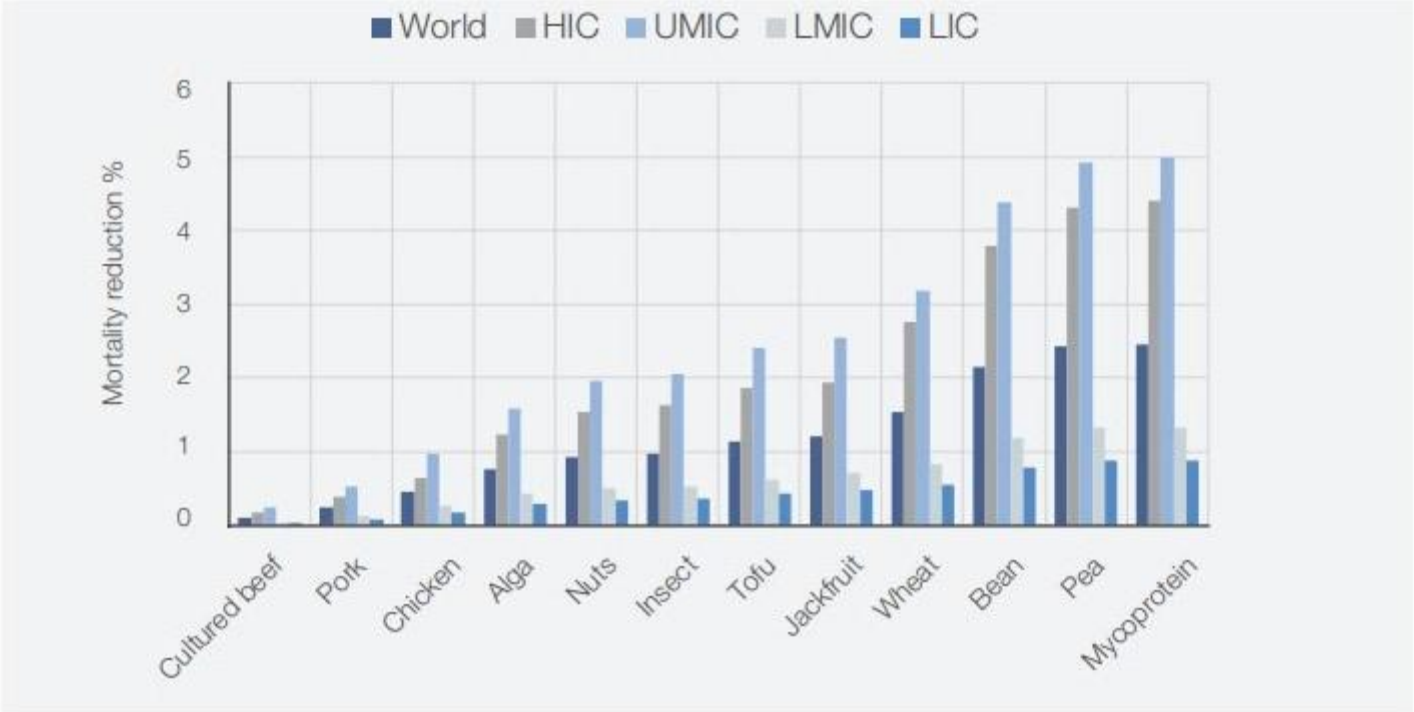
Sources: Data from FAO, IFAD, UNICEF, WFP and WHO, "The State of Food Security and Nutrition in the World 2018: Building Climate Resilience for Food Security and Nutrition", 2018, http://www.fao.org/3/I9553EN/i9553en.pdf; Development Initiatives Poverty Research Ltd, Global Nutrition Report 2017: Nourishing the SDGs, 2017, https://reliefweb.int/sites/reliefweb.int/files/resources/Report_2017.pdf.

Nutritional divides among the world's 7.5 billion peopleImage: World Economic Forum

Illustrating how change will positively affect societal outcomes across multiple areas will enable a faster and smoother transition than, for instance, justifying a change strategy on environmental or climate action grounds alone.

According to new research conducted by the Oxford Martin School for the World Economic Forum, Alternative Proteins, balancing one's diet with alternative proteins can reduce diet-related mortality by up to 5% and result in reduced environmental impact at the same time.

Looking at emerging and developing economies, a report by the International Livestock Research Institute, also for the World Economic Forum, Options for the Livestock Sector in Developing and Emerging Economies to 2030 and Beyond, shows enhanced grassland management could sequester up to 150 megatonnes of CO2 equivalent annually, while promoting livelihoods and food security.



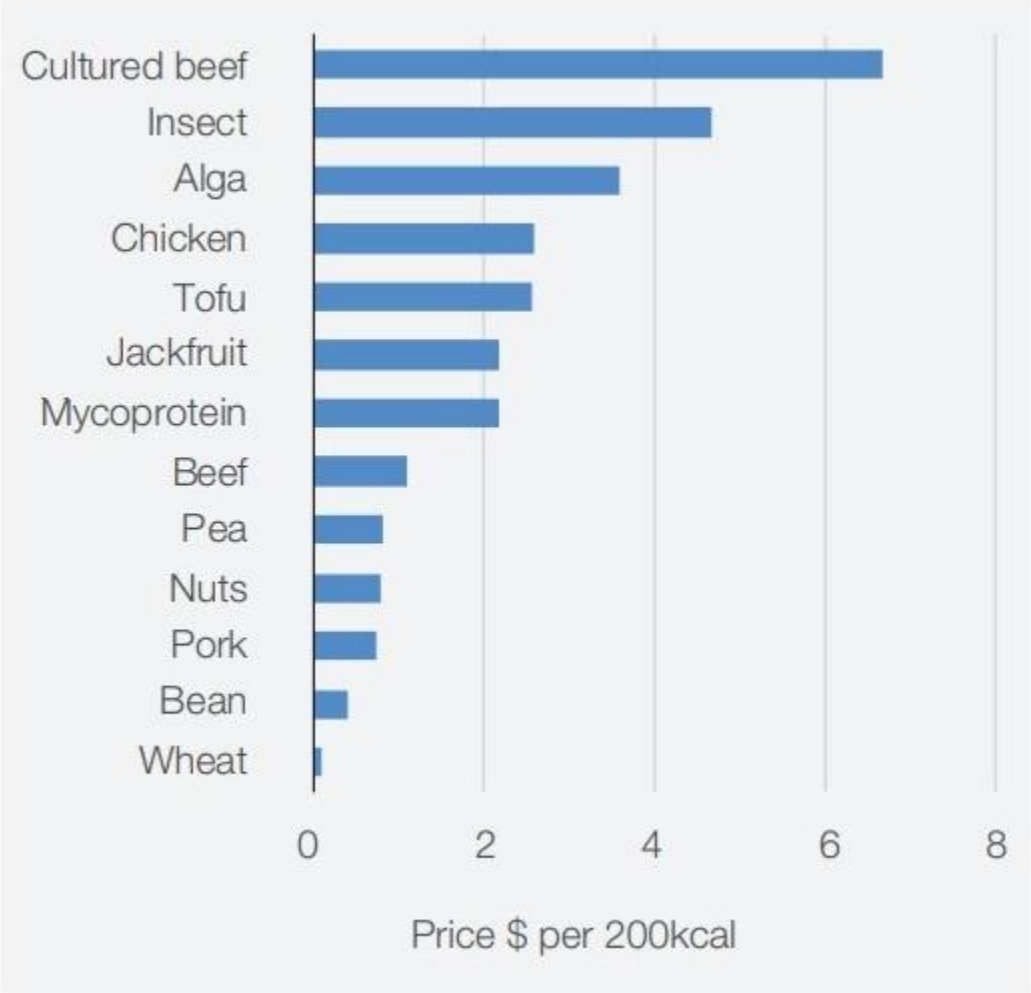
HIC: high-income country; UMIC: upper-middle-income country; LMIC: lower-middle-income country; LIC: lower-income country.

Net health effects of substituting beef with different food types globally and by national income classImage: World Economic Forum

Linking multiple positive agenda outcomes – or SDG targets – through one singular topic is a first step in helping key decision-makers and leaders understand the importance of this topic, and encouraging them to help drive change.

Promoting pathways to achieve cost parity

Cost is a central component in the consumer value equation. However, neither alternative proteins – such as the aforementioned Impossible Burger or lab-grown meats (not yet available to the market) – nor animal-based meat grown in a more sustainable manner are currently priced competitively with beef or pork. This means consumers are not necessarily incentivized to diversify their diets with these choices, nor are farmers incentivized to invest in more environmentally sustainable methods of meat production.



Estimated current prices of the different food typesImage: World Economic Forum

Subsidies could be one way to balance out pricing for alternative proteins and more sustainable production methods for traditional meat, and to help grow the markets in these two categories. There is a lot to be learned about innovation in the areas of cost and pricing – and the policy mechanisms that

can help promote 'tipping point change' – from the transition to clean energy. For example, in 1990, a German photovoltaic (PV) rooftop system cost roughly €14,000/kW. By the end of 2015, the price was less than one-tenth that amount at €1,300/kW. These results stemmed from early policy enablers in Germany that helped promote the scaled installation of PV. Because of these early efforts, scaled investment has occurred, and subsidies for renewables are now no longer needed. Could similar support for the evolving protein sector play a similar role in increasing availability at an accessible price point?

Pursuing an intentional transition decade from 2020-2030, leveraging narratives

The transition from meat-based proteins to a wider range of alternatives will play out in different ways around the world. However, a reliance on the market or a hope that individual technologies, unconnected projects, or even financing or policy innovations will cause a global breakthrough, even collectively, is perhaps optimistic. These will likely not be enough to create the scale or speed required to provide universally accessible and affordable, healthy and sustainable protein in line with the SDGs and 2015 Paris Climate Agreement targets by 2030.

This is because of the inherently personal and cultural nature of meat in human diets and livelihoods. The unique emotional and cultural politics of food, particularly of meat, mean that another key strategy to accelerate the transition will be required: narratives.

Common narratives – the stories we tell each other about food - can suggest what may be influencing the beliefs of both policymakers and individuals about where the benefits or risks of positively disrupting today's global protein provision system could lie. Starting from a common evidence base and framing the opportunities using narratives that are both honest and resonate with people's aspirations and needs will be essential in enabling an accelerated transition.

Take, for example, the recent $300 million Chinese investment in three Israeli-based start-ups working in the lab-grown meat sector. Technology and finance alone will not guarantee Chinese consumers will aspire to eat this form of protein, however. In this case, China - as the Oxford Martin research notes - has a significant public preoccupation with food safety, may benefit from narratives around the controlled environment in which cultured meat is grown to offset consumers' safety concerns.

Coordinated public-private efforts and intergovernmental engagement may well be required to develop and own both a global narrative on the protein transition, and specific regional narratives – such as in China – along the lines of how other global public health or education agendas are promoted. These efforts should be pursued over the next decade to help overcome the critical cultural and emotional barriers that may stand in the way of a holistic transformation.

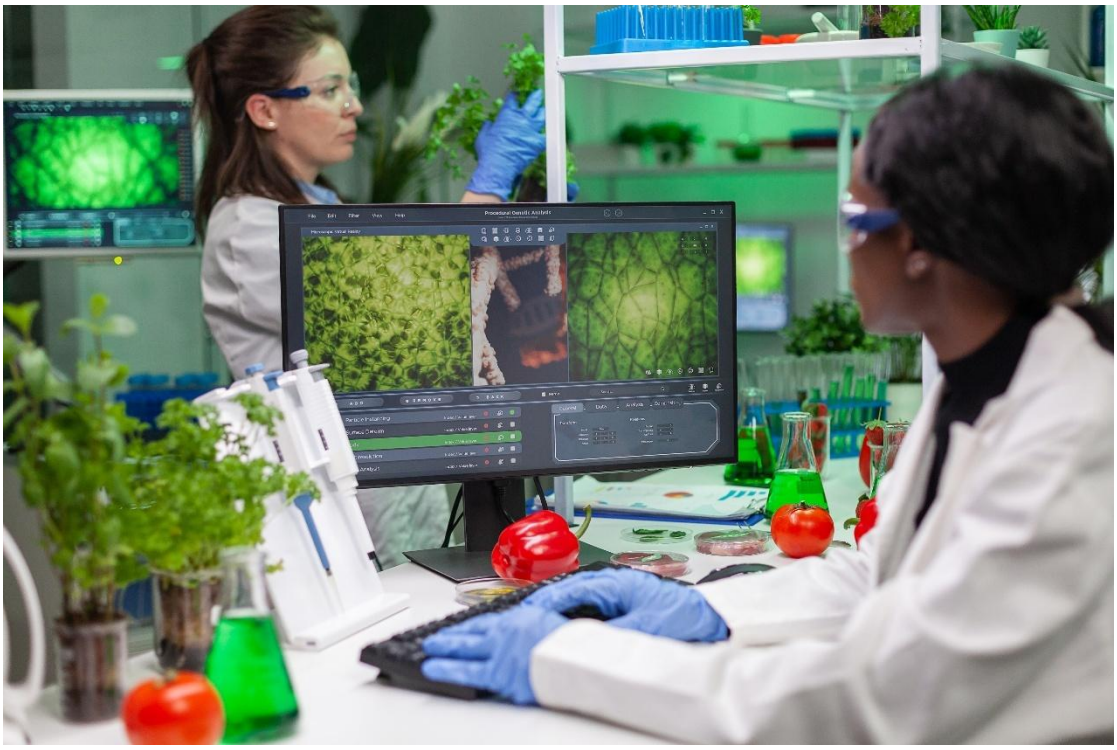
Developing a public-private accelerator for research, innovation, and scale

Finally, the discussions this year across four continents, combined with the aforementioned research findings, underscore the fact that the acceleration and scaling of various options within the global protein system – whether accelerating alternative protein, driving enhancements to today's current animal-based production systems, or shifting consumer behavior – is going to be a local affair when it comes to driving actions on the ground.

To do this, collaboration on a grand scale is necessary. New forms of public-private partnership will be required. Many stakeholders suggested that much can be learned from the creation and evolution of the Consultative Group for International Agricultural Research (CGIAR) system of networked research centers, created in the 1970s to help address the food crisis through advancing the green revolution in different regions around the world and through diversified, locally suited crops. Could a 21st-century variant on the CGIAR model, itself a public-private funded initiative supported by major philanthropic foundations, be developed: a consultative group for international protein options acceleration, for example?

Regardless of the form the next step takes, there is a lot to be done. The time to act is now if we are to provide universally accessible and affordable, healthy and sustainable protein in line with the SDGs and the Paris Agreement, as we approach a global population of 10 billion.

Is cellular agriculture the climate-friendly answer to growing food demands?



Cellular agriculture uses individual cells from plants and animals or single-cell organisms to make agricultural products.

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Cellular agriculture is poised to fulfil growing nutrition demands with minimal environmental impact. Further investment and commitment are required to scale up production and bring costs down. Transparent communication is needed to build trust and encourage consumer uptake.

The World Resources Institute estimates that global demand for beef and other ruminant meats could increase by 88% between 2010 and 2050, driven by a growing middle class and world population, which is expected to reach 10 billion by 2050. To meet overall nutrition requirements in the future, the Food and Agriculture Organization predicts that food production must increase by 70%. In parallel, consumer preferences are shifting as people make more deliberate food choices.

Cellular agriculture is a key pillar in the fast-paced “new food” sector, which also includes plant, microorganism, and insect-based alternatives. Positioned for rapid growth over the next decade, cellular agriculture will play an important role in fulfilling future nutrition requirements while taking pressure off current food production systems and the environment.

How does it work?

Cellular agriculture uses individual cells from plants and animals or single-cell organisms to make agricultural products. These include meats, seafood, dairy, and other protein-rich foods and functional ingredients, which are produced either through tissue engineering or precision fermentation without the need to “cultivate” entire animals or plants.

Tissue engineering is employed to make cell-based or cultivated meat, seafood, and milk. To produce cell-based meat and seafood, natural or genetically modified stem cells are taken from a live animal and grown in nutrient-rich conditions in a bioreactor, utilizing nature’s own growth and repair mechanisms. The cells differentiate into types – either muscle or fat cells – then are grown on a scaffold or further processed as ground meat.

Tissue engineering is employed to make cell-based cultivated products such as this salmon nigiri.

A similar method is used to produce cell-based milk. In this case, mammal milk gland cells are immobilized in a hollow fiber bioreactor. As a result, the cells secrete whole milk, which has the same macronutrient profile as cow or human breast milk, depending on the cell source.

Precision fermentation extends well-established methods widely used by the food and pharmaceutical industries. The process involves taking the gene that encodes, for instance, a target protein from a donor organism, such as a cow, and inserting it into the DNA of a host. The host, often a single-cell organism,

like bacteria or yeast, is cultivated in a fermentation tank, causing it to produce the target in large quantities. The resulting protein is separated from the host cells, purified, and typically dried to create a powder, which can be used as a sweetener, an ingredient in dairy-based ice cream, egg white protein, or collagen, for example.

Similarly, precision fermentation is utilized in the production of plant components, such as soy heme. Here, DNA is taken from a soy plant and inserted into a yeast via gene engineering. Following fermentation, the soy heme, which is similar to animal heme, can be used in meat analogues to impart a red or pinkish color, a meat-like texture, and taste.

The same method is applicable for making enzymes, silk, and leather, which are also protein-based, or to create non-protein ingredients such as fats or human milk oligosaccharides for breast milk substitute.

What are the advantages?

Cellular agriculture has the potential to provide the nutrition and other non-food products our growing population requires, without encroaching on additional lands or further stretching our natural resources. Because the production processes occur within a controlled environment and are largely based on established technologies, the benefits are wide-reaching. Cellular agriculture foods:

- Have a high feed conversion ratio and deliver similar or identical nutrition profiles.
- Meet high standards of consistency, safety, and hygiene.
- Ensure increased food security given independence from seasonal and climatic changes.
- Avoid animal antibiotics, thus minimizing antimicrobial resistance (AMR).
- Allow for selection of cell lines from animals with the best traits or from hard-to-culture species or those facing extinction.

What are the challenges?

Achieving industrial-scale capacity and price parity for cellular agriculture products requires overcoming specific hurdles.

For tissue engineering, this includes:

- Lowering material costs by using precision fermentation to produce culture media components (e.g., peptides), recycling media, and using less demanding cell lines.
- Increasing production efficiency by designing fit-for-purpose bioreactors that are large and scalable yet minimize cell damage.

For precision fermentation, this includes:

- Improving host productivity and efficiency.
- Reducing sterility requirements to lower production costs.
- Recycling metabolic heat and capturing CO2 for reuse to increase energy efficiency and minimize GHG emissions.

How can we maximize it?

Achieving the full potential of cellular agriculture will take decades; however, there are steps we can take to gain momentum. One is to start going after low-hanging fruit, such as pre-approved yeast strains in precision fermentation processes. Another is hybrid products, which combine cell-based components with plant-based foods, improving the taste, texture, and nutrition of the latter. Also, fungi and plant-based scaffolds can be used as supports to grow muscle cells, reducing production costs.

On a macro level, regulatory agencies should have discussions now about what is required to bring new foods to market before applications arrive. This would be beneficial to all stakeholders and improve the efficiency and speed with which products enter the market. Another obvious and important factor is increased investment in start-ups and companies focused on cellular agriculture to speed up R&D and scale up production – and of course, ensuring renewable energies are utilized throughout.

Lastly, building consumer trust is imperative for these products to gain traction. This requires transparent and regular communication about how these products are made and how they relate to foods and medicines that people already enjoy and depend on daily – whether that's cheese or insulin. A forecast from McKinsey & Co sees cultivated meat, for example, achieving cost parity with conventionally produced meat by 2030. That means the time to have these discussions is now.

Transition to cellular agriculture reduces agricultural land use and greenhouse gas emissions, but increases demand for critical materials

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Abstract

Cellular agriculture, that is, the production of cultured meat and microbial proteins, has been developed to provide food security for a growing world population. The use of green energy technologies is recommended to ensure the sustainability of changing traditional agriculture to a cellular one. Here, we use a global dynamic model and life-cycle assessment to analyze scenarios for replacing traditional livestock products with cellular agriculture from 2020 to 2050. Our findings indicate that a transition to cellular agriculture by 2050 could reduce annual greenhouse gas emissions by 52%, compared to current agriculture emissions, reduce demand for phosphorus by 53%, and use 83% less land than traditional agriculture. A maximum 72% replacement of livestock products with cellular agriculture using renewable energy is possible based on the 2050 regional green energy capacities. A complete transition can be achieved but requires 33% of the global green energy capacities in 2050. Further, the accelerated demand for critical materials will not exceed their primary production capacities, except for tellurium. We conclude that a transition to cellular agriculture is possible with environmental benefits and provide a benchmark to study different alternatives to animal-based diets.

Introduction

The world is heading towards unprecedented levels of global warming, partially driven by intensive livestock production, which causes 20% of global anthropogenic greenhouse gas (GHG) emissions[1]. We also need to produce around 40% more livestock proteins to meet the global demand in 2050[2]. Therefore, food and agriculture industries have been seeking novel alternatives, such as cellular agriculture, as an emerging branch of biotechnology to mitigate the environmental and animal welfare challenges related to conventional animal farming for meat and dairy production[3]. Previous studies have shown that protein-rich foods produced by cellular agriculture (e.g., cultured meat and microbial proteins) can be a solution for the problems related to industrial livestock farming by overcoming some of its undesirable consequences, such as global warming, land, and water use[4,5,6]. However, cellular agriculture is energy-intensive and might increase industrial energy consumption as it replaces biological systems with chemical and mechanical ones. Hence, using low-carbon energy technologies for cellular agriculture is necessary to maintain the initial objective of decarbonized food systems. This is in line with the global roadmap initiative where 'green energy' technologies, e.g., wind and solar-based power, are estimated to make up around two-thirds of worldwide energy production by 2050[8]. It is estimated that the share of electricity in global energy consumption will increase from 19% in 2016 to 49% in 2050, and the share of renewable energy sources will increase from 24% to 86% during the same time period.

Agricultural production, cell-culturing technologies, and green energy generation use raw materials that serve nutritional, structural, and technology-specific purposes. Some of those materials possess very high economic importance, are under high supply risk, and are vulnerable to supply restrictions. Thus, they are classified as critical materials, based on the United States Geological Survey (USGS) and the European Union (EU) Commission listings. The rapid growth of technological innovations has accelerated the demand for critical materials. This positive correlation between the two phenomena (technological innovations and material usage) has uncovered challenges of sustainability for critical material supply chains[14]. Hence, the

causal relationship between cellular agriculture practices, green energy technologies, and the consumption of critical materials needs to be investigated. This study is driven by a question: to what extent can cell-cultured foods produced with green energy meet global demand for meat and dairy proteins without exceeding the maximum extraction rates of critical material reserves? In addition, what is the impact of this transition on global GHG emissions, agricultural land, and phosphorus consumption?

Here, we assess the availability of critical materials for technologies used in green energy sectors, particularly for cellular agriculture. Nutrient inputs are essential during cell-culturing for protein production to provide final products with nutritional benefits comparable to livestock commodities. Phosphorus (P) is an essential nutrient for food and human consumption[18]. In the green energy sector, critical materials are needed for constructing steel, i.e., chromium (Cr), nickel (Ni), and equipment, such as aluminum (Al), zinc (Zn), and manganese (Mn)[10]. For wind power technologies, turbines are heavily reliant on permanent magnets made of critical materials, including mainly boron (B), and rare earth elements (REE), i.e., terbium (Tb), dysprosium (Dy), neodymium (Nd), and praseodymium (Pr)[19]. For solar photovoltaic (PV) panels, silicon (Si), germanium (Ge), gallium (Ga), tellurium (Te), and indium (In) are among the critical materials used for building solar cells, depending on the type and technology[20]. Cellular agriculture production is heavily dependent on stainless steel–based facilities for processing tanks[21]. The stainless-steel structure of the equipment helps to prolong the lifetime of the equipment and prevent corrosion. Stainless steel is a common alloy used in dairy production and wastewater treatment facilities as well. Common steel grades used for these applications include 316 and 304 steel, which contain two critical materials, i.e., Cr and Ni[22,23].

This work provided a global assessment of the impact of cellular agriculture on the consumption and the resource capacity of materials such as P, Al, B, Cr, Zn, Ni, Si, Te, Ge, In, Ga, Mn, Tb, Dy, Nd, and Pr. In this study, the primary production capacity refers to the maximum probable output of the extracted material given current facilities and extraction technologies[24]. The concept of maximum production capacity represents the upper limit of primary production of raw materials that the global industry can achieve without

incurring additional costs or making changes to its existing infrastructure. We simulated the food system transition from traditional to cellular agriculture and assessed the application of the most common green energy technologies (i.e., wind turbines and photovoltaic panels) in this transition.

The model design followed the system dynamics approach to understand the complexity of systems integration, i.e., food production, energy supply, agricultural land use, GHG emissions, and demand for critical materials. The model is explicitly described in the methods section as well as Supplementary Method 1, Supplementary Tables 3 and 4.

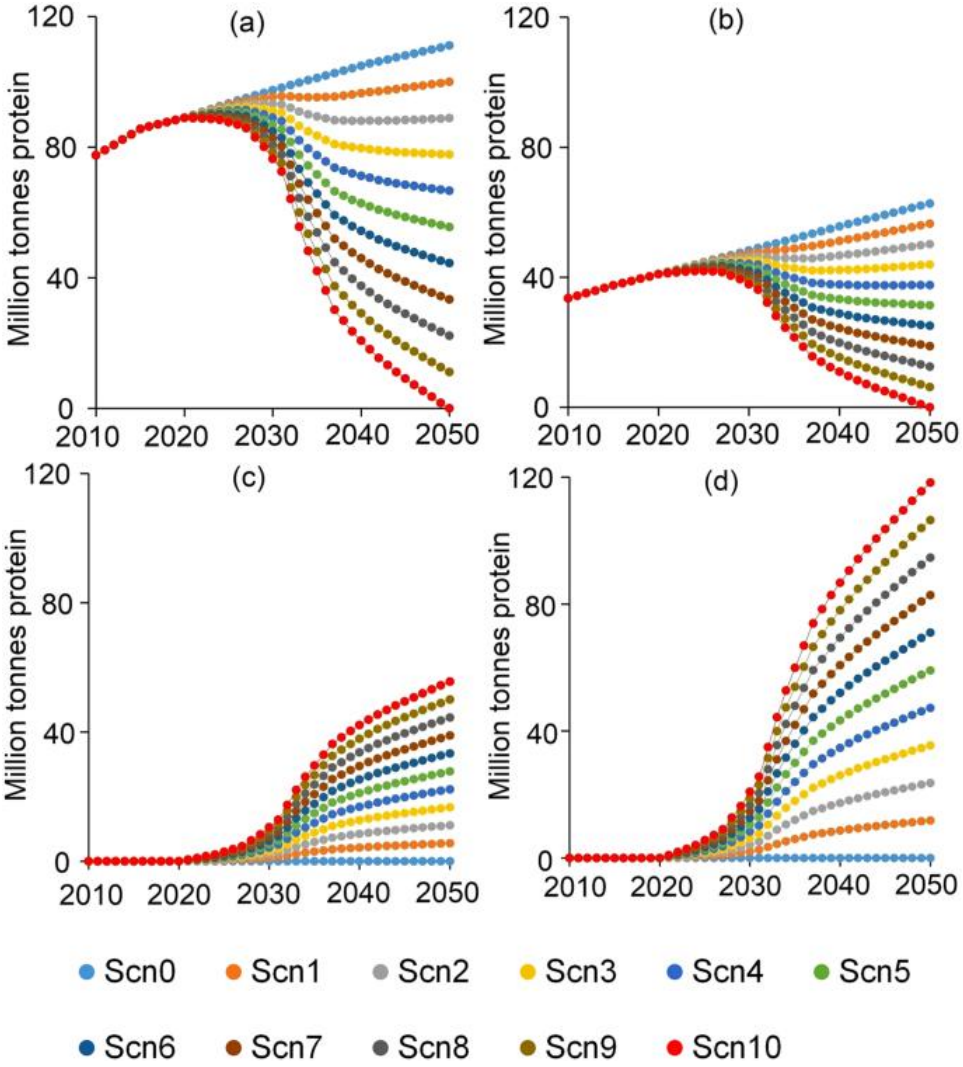
Cellular agriculture refers to culturing animal, plant, or microbial cells in bioreactors to produce alternatives to agricultural products[25]. Microbial protein (MP) and cell-cultured recombinant proteins (RP) are cellular agriculture products that can be used as substitutes for animal-based proteins in human diets.

We analyzed the application of MP and cell-cultured RP as alternative protein sources. The detailed production processes were considered based on the study by Järviö et al.[29] for MP from hydrogen-oxidizing bacteria (Supplementary Data 12) and Järviö et al.[30] for cell-cultured RP using "Trichoderma reesei" (Supplementary Data 7 and 8).

The transition is intended to replace livestock products. MP replaced meat varieties, including cattle, pork, lamb, and poultry, as they deliver high-quality protein comparable to livestock meat proteins[31]. Whereas cell-cultured RPs replaced eggs, dairy (i.e., milk and cheese), and meat varieties as they can deliver the nutritional benefits of egg ovalbumen, dairy proteins, and meat proteins[28]. The transition model to cellular agriculture considered the replacement of livestock products with microbial and cell-cultured recombinant proteins based on the protein contents of commodities. The calculations of the replacement and the protein contents of livestock commodities (i.e., beef, pork, lamb, poultry, milk, cheese, and eggs) can be found in Supplementary Tables 3 and 4.

The scenario analysis was conducted to assess the global transition to cellular agriculture within 30 years (2020–2050), which aligns with the global road-map initiative for a zero-carbon environment[8]. The transition to the studied cellular agriculture products was based on the S-curve gradual adoption process, where key players include early adopters, early majority, late majority, and laggards. This process reflects how the consumers perceive novel products entering the market (i.e., early adopts are risk takers, late adopts are risk-averse)[33]. The adoption process of the model fades in from 2020 until reaching the saturation stage by 2050. Data on the designed adoption can be found in the annotated code, Supplementary Methods 6 and 7. Keeping in mind that the future production of livestock proteins is influenced by population, income, and livestock demand[2], we analyzed several scenarios. Starting from a reference scenario where there is no replacement taking place (Scn0), we developed ten replacement scenarios reaching a 100% replacement of livestock with cellular agriculture (Scn10)—a 10% incremental increase between each two scenarios (Fig. 1a–d). We referred to Humpenöder et al.[2] in designing the replacement percentages of livestock with cellular agriculture. Humpenöder et al.[2] assumed a maximum of 80% replacement of ruminant meat with sugar-based microbial proteins. In this study, we aimed for the full replacement of livestock in order to provide the maximum spectrum of changes that can occur in the future.

Fig. 1: Global meat, dairy, and egg production (in million tonnes protein) under different replacement scenarios (Scn0→Scn10) between 2010 and 2050.



a Livestock meat production. **b** Livestock dairy and egg production. **c** MP production. **d** Cell-cultured RP production.

This research showed the environmental benefits associated with the global replacement of livestock with the studied cellular agriculture products, i.e., MP and cell-cultured RP, powered by green energy technologies. The benefits were characterized by reducing the overall GHG emissions and the agricultural land use associated with the food chain. Moreover, the results indicated a sufficient global capacity of green energy technologies in powering this

cellular transition, at least until 2050. Despite the consequences of the cellular agriculture transition on increasing the demand for the associated critical materials, we demonstrated the possibility of such a transition happening within the global budgets of the studied critical materials. Tellurium was the only exception, where the primary production capacity limits did not allow for >60% global livestock replacement.

Results

Trends in energy consumption in the food system

The energy system assessment covered the demand for critical materials following the changes in green energy supply and facility production, as the system analysis followed the cradle-to-gate approach for all food and cellular agriculture products, including the raw materials needed for the energy supply and facility production (Supplementary Fig. 1).

The energy required for cellular agriculture includes 'direct' and 'indirect' energy inputs. The production processes and the related environmental data of cellular agriculture products (i.e., microbial proteins and cell-cultured recombinant proteins), including GHG emissions and direct/indirect energy consumption, were derived from life-cycle assessment (LCA) studies by Järviö et al.[29,30]. Direct energy refers to the electricity needed for water electrolysis, propagation, fermentation, pasteurization, separation, and drying of the microbial protein product[29]. For cell-cultured RP, direct energy refers to the electricity needed for cultivation, filtration, purification, and drying of the cultured recombinant protein product, in addition to the electricity required for cleaning[30].

Indirect energy includes all the energy needed to produce the raw materials and resources for product manufacturing[34]. For cell-cultured RP, the indirect energy is used to produce ammonia water, ammonium sulfate, salt mix, antifoaming agent, glucose, and the resources for the electricity used for unit production. For MP, the indirect energy is used to produce nutrients, ammonia water, and the resources for the electricity used for unit production.

Our results showed that the transition to cellular agriculture by 2050 increased the energy demand of global food systems by 69%, reaching around 14.6 petawatt-hours when wind energy was used, and 83% reaching around 15.8 petawatt-hours when solar PV energy was used for the Scn10 replacement scenario. Around 52–56% of the energy demand originated from MP and cell-cultured RP production (Supplementary Discussion 1, Supplementary Fig. 3). The utilization of either wind or solar PV sources to power the global transition to cellular agriculture leads to different energy demands of the global food system. This was driven by the different indirect energy inputs needed to generate each energy source (i.e., wind and solar PV), where solar PV requires 1.3 times more energy than wind per kWh generated.

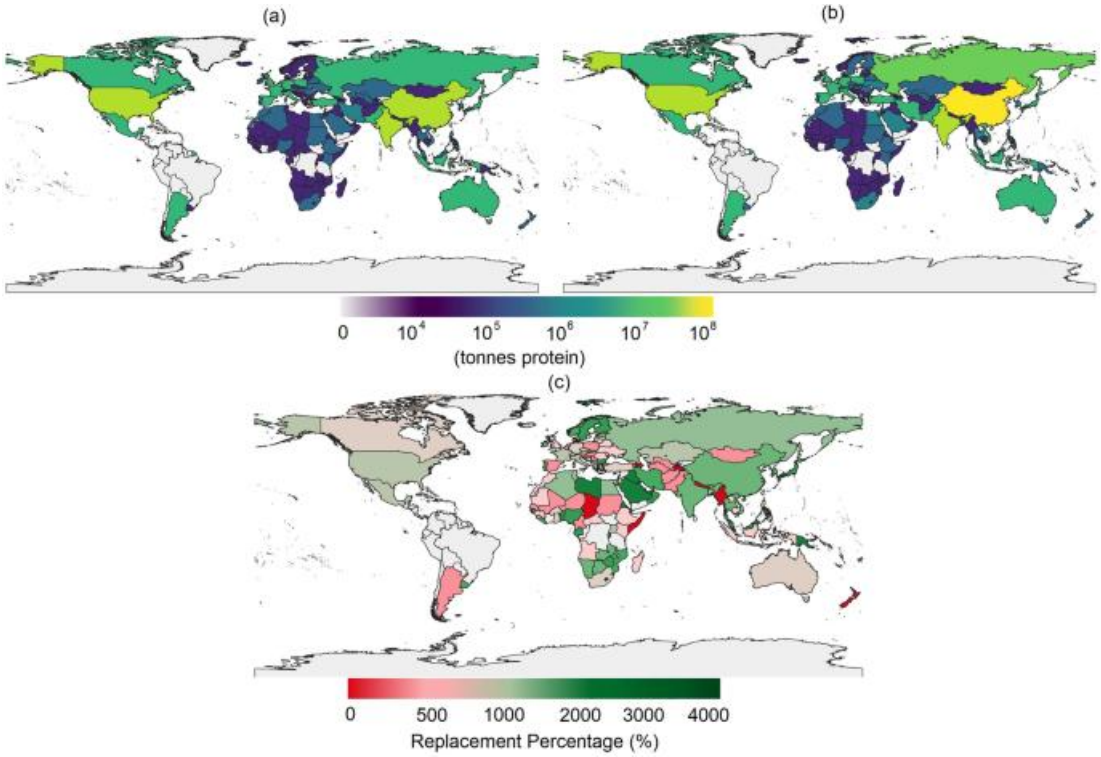
Deployment of green energy technologies

In this work, the maximum power output of energy sources is called "energy capacity". The allocation of green energy capacities to fuel the global transition to cellular agriculture is limited by several factors, such as physical obstacles (e.g., Betz limit for wind turbines or huge surfaces required for solar panels), technical barriers (e.g., shortage of critical materials), and social constraints (e.g., affordability of products or changing consumption patterns). We referred to current global capacities derived from the latest report of British Petroleum (BP)[35], in addition to the latest work by Carrara et al.[10], where three future demand scenarios were developed for green energy capacities by the end of 2050, namely: Low- (LDS), Middle- (MDS), and High-demand (HDS) scenarios. The development of these scenarios is based on four major factors: (1) Power generation capacities, (2) Plant lifetime, (3) Sub-technology market shares, and (4) Material intensity. Wind and solar-based energy technologies are considered in the analysis: onshore and offshore turbines for wind-based energy as well as crystalline silicon (c-Si), cadmium telluride (CdTe), copper indium gallium diselenide (CIGS), and amorphous silicon (a-Si) technologies for PV.

Figure 2 represents national and global energy capacity-based analysis of cellular agriculture transition in 2050 (Details on country-level livestock production and local green energy capacities can be found in Supplementary Data 1 and 3). The objective is to determine the regions with the highest

potential to lead the way for the transition to cellular agriculture based on the world's share of green energy capacities. Considering the national capacity of energy in 2050, if each country wants to satisfy its demand for cellular proteins, there is a production limit of the required green energy (Fig. 2a). Assessment of the required energy for national cellular agriculture activities shows that a maximum 72% of livestock products can be replaced by cellular proteins in 2050, using wind turbines (37%) and solar PV panels (35%). Around 63% of this transition takes place in Europe, North America, and Northeast Asia (Fig. 2a). The results reflect the unequal distribution of green energy resources, where Europe, Northeast Asia, and North America possess around 70% of wind capacities, while South Asia, East Asia, and North America possess 76% of solar PV capacities worldwide.

Fig. 2: Projection of the global production of cellular agriculture to replace livestock proteins in 2050 under the 2050 green energy capacity levels.



Light grey areas correspond to countries with no available data. Each country aims to replace its own livestock protein production with cellular agriculture proteins as much as possible, using local green energy. **b** Each country aims to

maximize the production of cellular agriculture proteins based on the availability of local green energy, where extra-produced cellular agriculture proteins go for exports. **c** Maximum replacement percentage of local livestock production based on the availability of local green energy. Replacement of livestock proteins includes meat (cattle, poultry, pork, and lamb), dairy (milk and cheese), and eggs.

Considering global energy capacity, as shown in Fig. 2b, a complete cellular agriculture transition (Scn10) can be achieved by 2050, which requires at most 33% of the global green energy capacities in 2050 (equivalent to 2120 GW and 2500 GW for wind turbines and PVs, respectively, for the lowest energy projection scenario). The potential increase in the transition percentage by a margin of 27% indicates the capacity of most regions to replace >100% of local livestock production, where extracellular products are intended for countries with less than 100% replacement percentage (Fig. 2c).

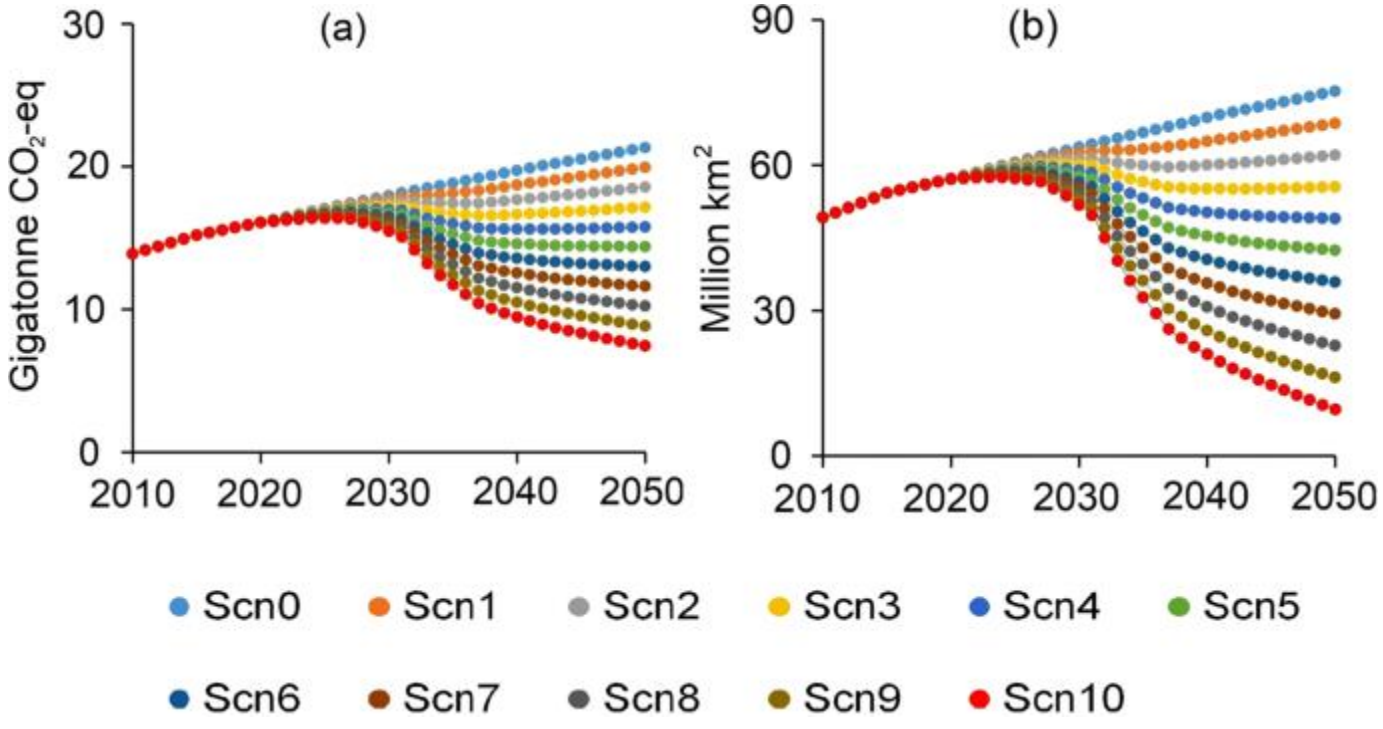
The growth in green energy capacities can facilitate higher transition rates to cellular agriculture. Wind and solar energy capacity levels are estimated to reach 2100–2500 GW under LDS, 3400–4500 GW under MDS, and 7900–12500 GW under HDS by the year 2050[10] (Supplementary Data 2). Considering all the studied projected world green energy demand scenarios, the development of green energy capacities allows for a full transition to cellular agriculture production by 2050, where wind or solar PV can be solely utilized for the energy supply system (Supplementary Discussion 2, Supplementary Fig. 4), requiring at most 1530 GW and 1265 GW of wind and solar PV capacities, respectively. The provision of additional energy for cellular agriculture from either fully solar PV or fully wind technologies allows us to assess the maximal impact on GHG emissions, agricultural land use, and demand for critical materials. By 2050, 72% (in LDS), 44% (in MDS), and 20% (in HDS) of global wind capacity will be required (Supplementary Fig. 4a–c). For the solar PV panels, 51% (in LDS), 28% (in MDS), and 10% (in HDS) of global solar capacity will be required (Supplementary Fig. 4d–f).

GHG emissions and land used in agriculture production

The assessment of GHG emissions and agricultural land use for all food commodities followed the cradle-to-gate approach with data derived from Poore and Nemecek[36], World Food LCA[37], Agri-footprint[38], and Järviö et al.[29,30]. The work by Poore and Nemecek[36] used the global mean data of the environmental impacts. This included 570 studies across 119 countries.

The results demonstrate that the cellular agriculture transition powered by either wind or solar energy sources reduces annual GHG emissions despite the increase in the overall energy demand. A full transition (Scn10) reduces the annual GHG emissions from the food system by 52%, reaching ~7.4 gigatonnes CO_2-eq in 2050 (Fig. 3a) compared with current annual emissions. This accounts for around 48% of current agricultural emissions. The progression in transition rates decelerates the growth of the cumulative GHG emissions, saving up to 132 gigatonnes CO_2-eq by 2050 under the Scn10 scenario, equivalent to 19% of cumulative emissions by the same year under the Scn0 scenario. Details on the GHG calculations can be found in Supplementary Data 15.

Fig. 3: Environmental impact of the global transition to cellular agriculture under different replacement scenarios (Scn0→Scn10) between 2010 and 2050.



Annual greenhouse gas (GHG) emissions from the food system. **b** Total agricultural land use from the food system.

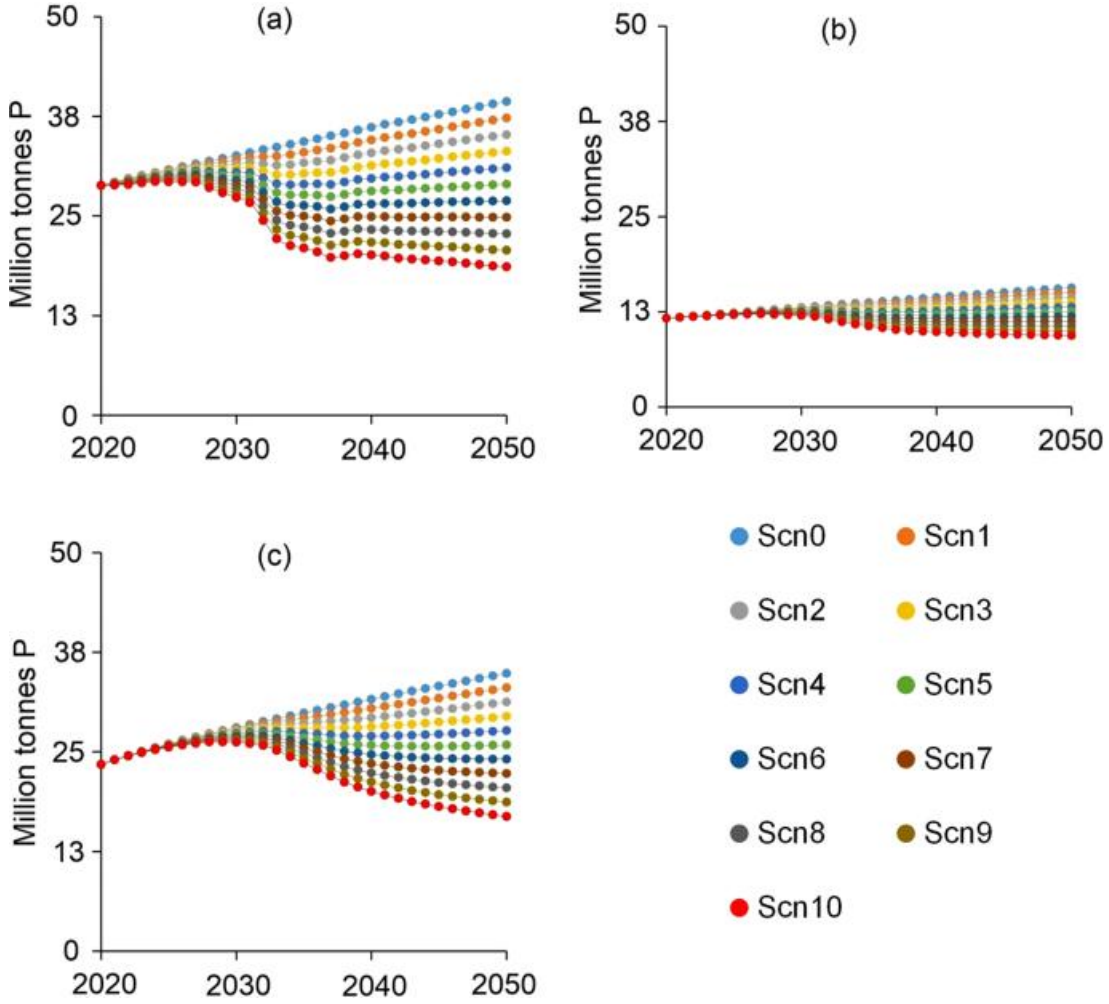
The transition to cellular agriculture would reduce the agricultural land requirements by 83% by 2050, releasing around 9.6 million km^2 of agricultural land for other uses (Fig. 3b). Land saving is mainly due to the elimination of pasture and grass used for livestock grazing that accounts for 84% of total agriculture land used for livestock production, in addition to crops production needed for livestock farming (Supplementary Fig. 6a, b). Pastures occupy more than 74% of total agricultural land in Scn0, while the rest is arable land. In Scn10, arable lands become the main contributor with 100% of total agricultural land in 2050 (Supplementary Fig. 6c, d). Cellular agriculture requires crop input—particularly cell-cultured RP—as a glucose source[30]. For the cell-cultured RP, the carbon source was assumed to be glucose produced from maize. The microbial protein production does not require agricultural land, as the hydrogen-oxidizing bacteria use CO_2 as a source of carbon. The overall results showed lower demands for maize when cellular agriculture replaced livestock production despite the need for maize inputs to the cell-cultured RP production, reaching 883 million tonnes by 2050 in Scn10, down from 1.2 billion tonnes in 2020. The decline in the overall maize demand was driven by the elimination of livestock production, which generally requires 65% of world maize production as animal feed. The land requirements for glucose production contributed up to 6% of the total arable land area by 2050 in Scn10. Details on the land use calculations can be found in Supplementary Data 20.

Trends in phosphorus flows

The transition to cellular agriculture reduces overall phosphorus consumption in the food value chain. Despite the comparable nutrient fractions in livestock and cellular agriculture products, the livestock chain experiences several spots of P losses, unlike cellular agriculture production, which is conducted in a precise and controlled environment. The transition to cellular agriculture reduces the primary production of phosphate rock by up to 53% (Fig. 4a). Crop production and nutrient loss are the main flows affected by the changes in

livestock production. The feed used for livestock production accounts for around 41% of the total cereals produced annually[40]. Hence, total crop production is directly affected by declining demand from the livestock sector. Considering a total replacement scenario, total crop production will decrease by 40% in 2050 (Fig. 4b). Along with the food supply chain, the major losses of nutrients primarily originating from agricultural runoff and livestock waste account for around 47% of the total phosphorus loss. The replacement of livestock with cellular products eases the pressure on cereal production, reducing the magnitude of P losses through waste flows by 51% in 2050 (Fig. 4c).

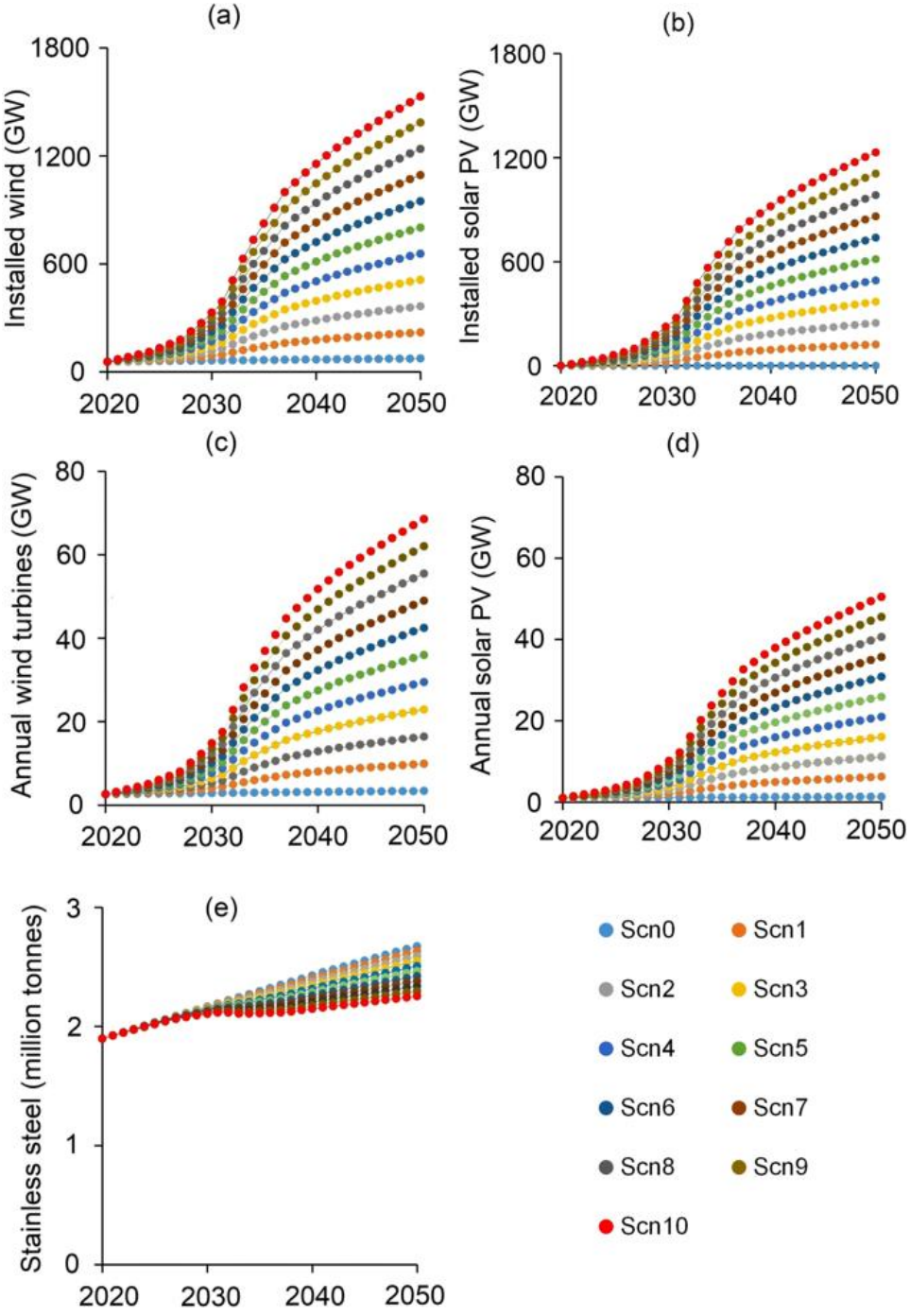
Fig. 4: Global phosphorus flows in a million tonnes P under different replacement scenarios (Scn0→Scn10) between 2020 and 2050.



a primary production, **b** crop production, and **c** P losses. Demand for green technologies and stainless steel.

Following the transition to cellular agriculture, the global demand rises for wind turbines and solar PV panels for green energy generation in the analyzed system, while the growth of stainless-steel demand follows a decline phase by 2050 (Fig. 5).

Fig. 5: Global resource requirements for the food system under different replacement scenarios (Scn0→Scn10) between 2020 and 2050.



a Installed capacity of wind turbines. **b** Installed capacity of solar PV panels. **c** Global annual production of wind turbines. **d** Global annual production of solar PV panels. **e** Global production of stainless-steel for cellular agriculture, dairy, and wastewater treatment facilities in the food system. The average capacity of a wind turbine is 2.75 MW (Supplementary Table 4). The average capacity of a solar PV panel is 315 watts (Supplementary Table 4).

In 2050, around 68.5 GW of wind energy is projected to be allocated to food production in the case of a 100% replacement scenario compared to 2.6 GW for a no-replacement one (Fig. 5c), reaching 1530 GW of cumulative wind capacity (Fig. 5a). Most of the used wind turbines are onshore, as they continue to dominate the wind power industry (over 88% of global wind energy capacity)[10]. Among the list of critical materials used for wind turbine installation in the analyzed system, zinc is the most common material (65% of total critical materials in turbines). This is due to the fact that zinc is used as a component of the coating materials, preventing turbine corrosion[41]. Aluminum, manganese, chromium, and nickel contributed to around 33% of the total critical materials applied in wind turbines, as they are used for structural purposes to strengthen turbine construction. Despite the projected decline of the needed structural critical materials due to the future optimization of wind turbines[42], these materials still constitute the largest share of critical materials to be used in wind turbines by 2050. The rest—predominantly REEs and boron—are used in much smaller amounts but are crucial to the operation of wind turbines using permanent magnets[17].

Focusing on solar power, the transition to cellular agriculture increases the demand for PV technologies, reaching up to an annual production of 50.47 GW in 2050, up from ~1 GW for a no-replacement scenario. This will lead to an overall installed capacity of 1265 GW (Fig. 5b). It is sufficient to ensure 100% replacement (Fig. 5d). The large gap between the demand levels of turbines and PV panels by cellular agriculture is driven by the amount of electricity to be provided by the specific technologies. Similar to the material intensity levels in wind turbines, structural materials, i.e., aluminum, nickel, and chromium, possess the largest shares of critical material input to the solar PV systems, which is equivalent to 90% of its mineral demand. Silicon is used due

to its electrical conductivity. It has the largest demand among the other critical materials, i.e., gallium, tellurium, germanium, and indium, for constructing solar PV systems. The technology of c-Si PV will continue to dominate the solar PV market (up to 95% of total shares) by 2050, despite the introduction of alternative PV technologies such as CdTe, CIGS, and a-Si[10].

The variations in installed capacities of wind and solar PV to power the global transition to cellular agriculture stem from multiple factors, i.e., discrepancies in the annual amount of electricity generated per wind turbine and solar PV panel, and the differences in the lifetime of those technologies. Details on the calculation of the annual capacity generation can be found in Supplementary Table 3.

Despite the demand for steel for MP and cultured RP production (e.g., bioreactors and machines for the downstream processes)[29,30], the minerals demand for stainless steel needed for food production (dairy and cellular agriculture) and wastewater treatment decreases in the food system if replacement scenarios take place (Fig. 5e). The use of stainless steel for wastewater facilities is the dominant driver of material consumption, accounting for 47% and 52% of total stainless-steel requirements in 2050 with 100% and 0% replacement scenarios, respectively. Stainless-steel consumption decreased by 16% followed by the replacement of dairy products, including milk and cheese, with cellular agriculture in Scn10. The annual total demand maintains the increasing trend, following the increase in steel wastewater reservoirs and piping systems to treat waste from human consumption, which is independent of the transition initiatives to cellular agriculture. Details on the wastewater treatment facilities can be found in Supplementary Table 4. Chromium and nickel are the major critical materials utilized in stainless steel used in dairy, wastewater treatment, and the biotechnological industry. The material intensity of those elements is driven by the grades of the steel used—304 and 316 stainless steel. The consumption of these materials per unit of steel input is not anticipated to experience any changes by the year 2050.

Critical material consumption as part of global flows

The demand for critical materials related to the transition to cellular agriculture is constrained by the global projections of nutrient flows, stainless steel production, and the development of wind and solar PV technologies. Global flows of the critical materials in the studied sectors can be found in Supplementary Data 4–6. The results demonstrate the possibility of achieving a total replacement of livestock products with cellular agriculture, powered solely by either wind or solar energy, without experiencing supply shortages by the year 2050 (Supplementary Fig. 7). The annual generation of global wind and solar-based energy will continue to grow to the year 2050, i.e., LDS by 70–100%, MDS by 200%, and HDS by 600%[10]. Similarly, the stainless-steel industry is anticipated to grow by 42% by 2050[43].

The demand for chromium and nickel in the analyzed system follows the same trend compared to the global trends in the stainless-steel industry. The flows of chromium and nickel in this system are related to stainless-steel products, where approximately 55% goes to the construction of solar PV and 44% to other sectors, including dairy production, wastewater treatment, and cellular agriculture production (Supplementary Fig. 7).

In a full replacement scenario, the amount of phosphorus needed for protein products will decrease by 10% in 2050, down from 60% of total availability in 2020 (Supplementary Fig. 7). The total availability corresponds to the available amount of phosphorus from all sources, i.e., primary production and recycling. The declining trend reflects the reduction of P losses that take place in the upstream level of the livestock value chain. This also indicates a more efficient chain of protein production via cellular products than current livestock practices, in which phosphorus demand for livestock production accounts for around 63% in 2050, given a no-replacement scenario. It should be noted that the phosphorus flows in the food system are independent of the different scenarios of green energy projections. Hence, no changes are observed with regard to phosphorus consumption under LDS, MDS, and HDS.

Given a total replacement powered solely by wind power technology, the critical elements (REEs, B, Zn, and Mn) used for the wind installations in the food system are estimated to cover up to 51% of total material flows to wind technology under LDS, 35% under MDS, and 15% under HDS by 2050

(Supplementary Fig. 7). These increases from 2% in 2020 follow the S-curve-based gradual adoption of cellular agriculture in the global food system. Similarly, the contribution of critical materials (Ga, Ge, In, Si, and Te) for the solar PV energy in the food system to the global flows increases as the total replacement takes place, accounting for 23% under LDS, 13% under MDS, and 6% under HDS by 2050. In the food system, aluminum is used predominantly in solar PV structures, with a percentage of around 85%, while the remaining aluminum demand is used for wind turbine structures. This explains the similar trends for aluminum and other materials used for solar PV in the global food system.

Demand for critical materials as a percentage of their global primary production

The demand for critical materials was studied to assess the impact of the cellular agriculture transition, accompanied by the use of green energy, on the global primary sources of critical materials. Results demonstrated the need for additional input of several critical materials ranging between 0.03% to 35% of current world primary production levels (Supplementary Fig. 8). Among the critical materials required for the wind energy sector, the demand percentage of REEs is the highest, including dysprosium, neodymium, praseodymium, and terbium, with increases reaching up to 9–17% of their primary production in 2050 for a total replacement scenario (Supplementary Fig. 8). REEs mining is strongly affected by the growth of green technology propagation, as a 1% increase in green energy capacities can cause 0.18% depletion of REE reserves[15]. The boron demand for wind turbines represents a small percentage of its global primary production. This is mainly due to the limited amount of boron used in wind energy systems[44]. The demand for structural materials in 2050, including aluminum, manganese, and zinc, increases and accounts for around 0.1%, 0.17%, and 1.9% of their total global primary production, respectively, given a total replacement scenario. The low demand percentages relative to the materials are due to the variety of purposes these structural materials can serve outside the energy industry. Around 90% of global manganese consumption is accounted for by the production of multiple steel-grade structures[45]. The use of manganese in the wind turbine industry is limited to the inclusion of steel to support the turbine structures, accounting

for around 1.6% of steel weight[46]. Despite the highest contribution of zinc to wind energy in the food system, among other critical materials, most mined zinc material is used in the construction sector in the form of zinc coatings for galvanized steel (>90%)[47]. Most of the produced aluminum is used for the building and construction sector, transportation, electronics, appliances, machinery, and equipment, accounting for more than 95% of total primary production[48].

The demand for key critical materials needed for solar PV in the food system is projected to increase by 2050, reaching up to 34% of the current global primary production of tellurium, given a total replacement scenario (Supplementary Fig. 8). The solar PV industry uses Tellurium heavily, comprising up to 40% of global production[49]. Similarly, germanium demand accounts for 19% of total germanium primary production, as most of the produced material is used in the semiconductor industry, particularly in the manufacturing of solar cells[50]. The demand for other key critical materials, i.e., gallium, indium, and silicon, accounts for lower percentages of their global production (1.6–21%). Despite the importance of silicon in the solar industry, where over 85% of current solar cells are based on silicon, >80% of silicon is used to produce silicone materials, superalloys, and cast iron[51]. The global primary production of gallium is often driven by the manufacturing of integrated circuits and laser diodes (>95%), while only <2% is used in the manufacturing of solar cells, mainly corresponding to CIGS technologies[52]. The global primary production of indium is mostly driven by the production of indium-containing alloys, compound semiconductor materials, and indium-tin oxides. The remainder is used for the manufacturing of CIGS cells for solar systems, accounting for less than 1% of global consumption[53].

When wind energy is used in cellular agriculture, the demand for chromium and nickel declines. The total elimination of livestock production, including dairy, will decrease the primary production demand of those minerals by 13% by 2050, considering the adoption of wind energy sources to the cellular agriculture transition activities. On the other hand, the demand for chromium and nickel in 2050 will increase, reaching up to 2% and 23% of their global primary productions, respectively, when solar energy sources are adopted in order to achieve a complete transition to cellular agriculture. These increases

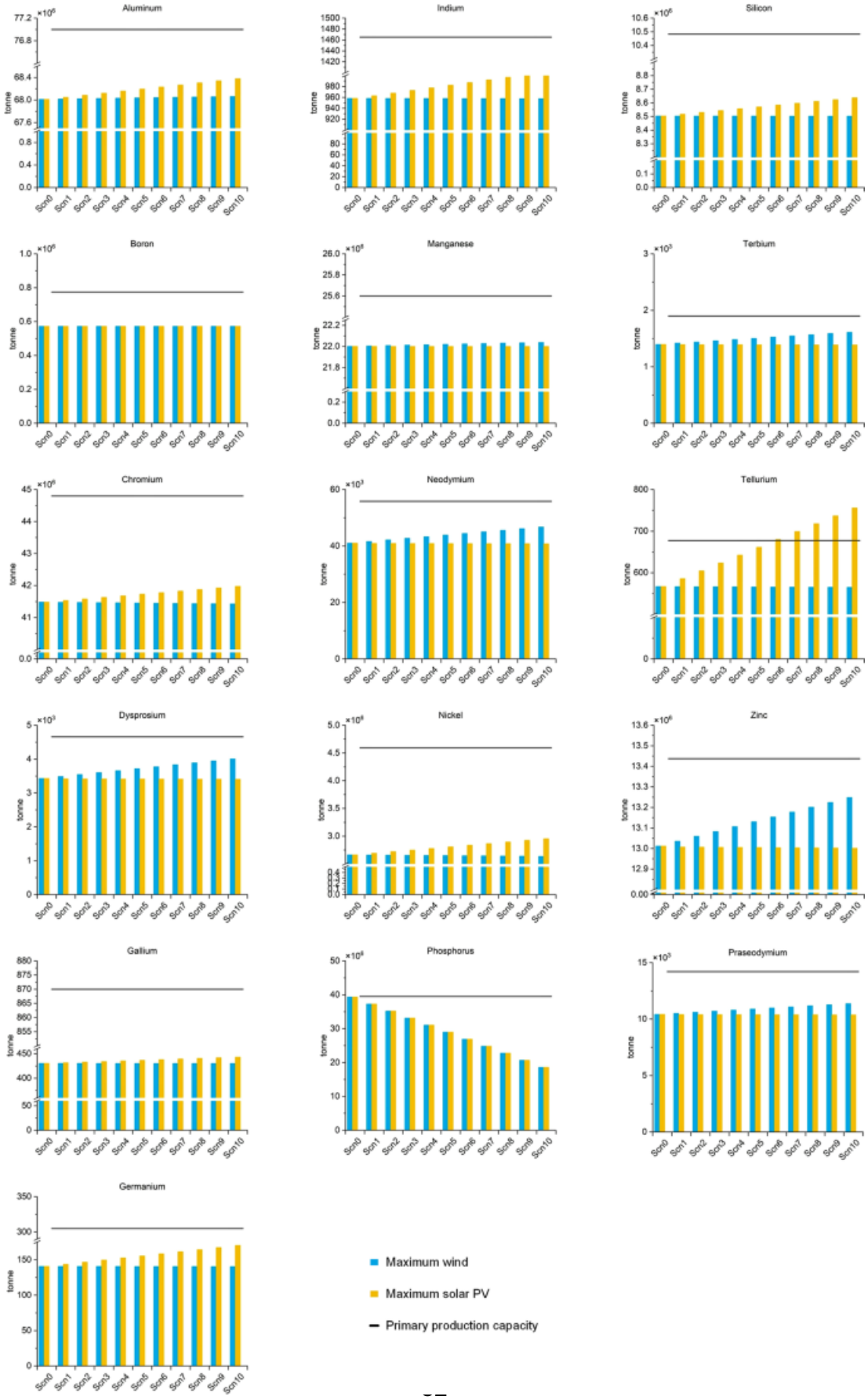
are driven by 14% higher material intensity of stainless steel in solar panels than in the dairy industry.

The primary production of phosphate rock—the elemental form of phosphorus—is increasingly driven by the growth of population and changes in lifestyle[18], reaching up to 1.3 times the current primary production by 2050, given current livestock production practices (Supplementary Fig. 8).

Demand for critical materials versus their primary production capacity

The level of demand for critical materials as a result of the transition to cellular agriculture was analyzed with regard to the associated capacity of their production. The results indicate that despite the increase in critical material demand to achieve a full transition to cellular agriculture by 2050, a 100% transition can take place without exceeding the existing primary production capacity levels for most materials (Fig. 6).

Fig. 6: Primary production of critical materials in 2050 to meet the global demand including the global food system requirements under different replacement scenarios (Scn0→Scn10).


Aluminum
Indium
Silicon
Boron
Manganese
Terbium
Chromium
Neodymium
Tellurium
Dysprosium
Nickel
Zinc
Gallium
Phosphorus
Praseodymium
Germanium
tonne
Scn0
Scn1
Scn2
Scn3
Scn4
Scn5
Scn6
Scn7
Scn8
Scn9
Scn10
Maximum wind
Maximum solar PV
Primary production capacity

The blue bars correspond to the replacement solely powered by wind energy, the orange bars correspond to the replacement solely powered by solar PV, and the horizontal black line corresponds to the current primary production capacity level.

In the case of cellular agriculture transition powered solely by wind energy, the additional amount of mined materials is not anticipated to exceed the primary production capacity for any of the associated elements, i.e., aluminum, boron, dysprosium, neodymium, nickel, praseodymium, terbium, and zinc. On the other hand, the demand for tellurium, mainly driven by the generation of energy by solar PV, will lead to a 34% increase in the current primary production that allows a maximal 60% transition to cellular agriculture by 2050. The maximum capacity of Te primary production is around 673 tonnes. This finding highlights the need for PV technology improvement in use and recycling for critical materials recovery. With current livestock production practices, phosphorus primary production will reach its maximum capacity by 2050—39 million tonnes. It should be stressed that phosphorus is the only critical material for which a total transition to cellular agriculture can lead to a decrease in the demand below its primary production capacity, meaning a decrease of 53% by 2050.

The estimated primary production capacities of several critical materials will be higher than their world demand in 2050, including zinc (1%), chromium (6%), aluminum (13%), boron (35%), manganese (17%), indium (53%), silicon (21%), REEs (37%), nickel (55%), gallium (96%), and germanium (79%).

Sensitivity analysis

A sensitivity analysis was conducted to examine the spectrum of changes in the main results. Two parameters among the cellular agriculture production acted as the dependent variables: (1) electricity consumption with an input increase of 20%, and (2) glucose production with an input increase of 20%. Details on the sensitivity analysis modelling can be found in Supplementary Method 2. The inventory inputs can be found in Supplementary Data 9–11 for the cell-cultured RP, and Supplementary Data 13 for the MP. The results

showed limited impact and no change to overall conclusions. The increase in glucose inputs increased GHG emissions, agricultural land use, and the consumption of primary phosphorus by 1.3%, 1.2%, and 2.6%, respectively. Increasing the electricity inputs had the highest impact on the consumption of the studied critical materials, with a 19–20% increase in demand for wind turbine and solar PV-related materials. Nickel and chromium consumption increased by 11% following the increase in electricity inputs. With the condition of increasing the electricity consumption by 20%, the world demand for Te reaches the maximum capacity limits with a 50% transition to cellular agriculture by 2050. Details on the sensitivity analysis results can be found in Supplementary Data 14. To ensure the accuracy of the model, the comparison between the model and real-world data showed at least 95% compatibility, i.e., P primary production, fertilizers production, agriculture land use, and GHG emissions from food production. Details on the percentage accuracy calculations and annual results can be found in Supplementary Method 5.

Discussion

The findings proved the possibility of utilizing solely wind or solar PV technologies in the transition to cellular agriculture globally. However, the combination of these technologies to power the global transition to cellular agriculture could bring flexibility and optimize the consumption pattern of the required critical raw materials. In addition, combining both sources could overcome the potential limitations on the regional levels, i.e., national and regional capacity limits of wind and solar PV energy sources. The integrated approach will be promising for the sustainable development of cellular agriculture on a global scale.

In this work, the environmental data collected for livestock and crop production referred to the mean values reported in Poore and Nemecek[36]. The results of Poore and Nemecek[36] demonstrated the high range of impacts on the production of livestock and crop commodities. The beef herd had generally the highest range of impacts, with the 90th percentile being 5.25 times higher than the 10th percentile for GHG emissions, while the 90th percentile of land use was 8.8 times higher than the 10th percentile for the same product. This range of impacts contributes further to the uncertainty of

the environmental benefits of the global food system in light of the global transition to cellular agriculture.

Carbon dioxide (CO_2) emissions generally persist in the atmosphere for millennia, forming an accumulating stock without a clear removal process. On the other hand, other climate pollutant emissions have comparatively shorter life spans, i.e., methane (CH_4), despite the immediate environmental damage they cause in the short term[54]. When differentiating between the different gas types in the studied model, the overall CO_2 emissions followed an increasing trend following the replacement of livestock with cellular agriculture by the year 2050. On the contrary, CH_4 emissions experienced a decreasing trend following the gradual decline in livestock production activities by the year 2050 (Supplementary Discussion 3, Supplementary Fig. 5). Detailed calculations of the CH_4 and CO_2 emissions can be found in Supplementary Data 16–19. The 30-year period of the studied dynamic model (2020–2050) does not allow a conclusive statement on the long-term environmental costs or benefits of the livestock replacement model with cellular agriculture, as Lynch et al.[54] report a period of 50 years before emissions and removals of CH_4 are approximately balanced.

Our research focused on replacing livestock products as a source of protein, without considering the by-products of animal production (i.e., fat, leather, organs, pet food, medicine, lubricants, and chemicals). The elimination of the studied livestock's main products (i.e., dairy, eggs, and meat from the studied livestock species) from the protein production model and replacing them with cellular agriculture products leads to the elimination of livestock by-products. The impacts of by-product replacement on the environment (i.e., GHG emissions, land use, energy consumption) and resource consumption (i.e., critical materials) were not analyzed.

The protein structure of the cellular agriculture products, i.e., MP and cell-cultured RP, is close to the respective animal-based protein sources[55]. The studied products contain all nine essential amino acids required by the human body. The ingredient lists provided for the microbial proteins (MP) and cell-cultured recombinant proteins (RP) production show the existence of certain nutrients essential for the healthiness of the food products, i.e., sodium,

potassium, phosphorus, magnesium, sulfur, calcium, manganese, iron, zinc, and copper[29]. The comparison of the nutrient profile of the studied cellular agriculture products and the livestock sector (nutrition data derived from the USDA[56]) showed that livestock products had higher nutrient contents, e.g., beef contained at least five times higher iron quantities and at most 425 times higher zinc quantities than MP when comparison was done based on the protein content. Hence, the transition from livestock to the studied cellular agriculture products would require more changes in diets to ensure sufficient intake of all essential nutrients.

The final output of the studied cellular agriculture products is flour-like powder, which is different from the various livestock commodities, i.e., meat, dairy, and eggs. Therefore, the powder requires further processing and mixing with plant-based ingredients to make processed replacement products for livestock. The additional impacts of the processing were not considered in this study. However, this is in line with the system boundaries of the livestock products, as only the processes up to the farmgate were included.

This work presented the environmental and resource consequences of replacing livestock with cellular agriculture at a global level. The findings do not support the idea of recommending a specific diet to be followed based on the obtained results. This work did not examine the flexibility of switching to different food alternatives, i.e., cellular agricultural products. Previous research has discussed the inclusion of various food alternatives to replace livestock products, showing potential benefits regarding nutrition, environment, and accessibility[57,58]. The results of this work can be utilized in future research as a criterion to study the different replacement alternatives of livestock-based diets.

Despite the existence of discovered reserves, in large quantities for some materials, the development of mining activities often relies on other factors, i.e., technological advancement, market economy, and the associated costs, including energy and operating costs[59]. In addition, some materials are refined as by-products of other mined materials, i.e., tellurium, which is refined due to copper mining and extraction. Despite the economic importance of tellurium in the sectors studied in this work, such as the solar PV industry, the future

extraction of tellurium is often driven by the main purpose of obtaining copper and not based on the availability of tellurium reserves, where copper producers are less eager to invest in the recovery of additional tellurium[60]. Currently, the scaled recycling of some critical materials in the green energy sector is far from developed[61]. For example, the recycling rate of neodymium magnets used in wind turbines is still less than 1%[62]. With this level of uncertainty surrounding the estimations of the future availability of critical materials used in the green energy sector, this work considered the mine capacity, which is the maximum possible output of annual mined materials based on current economic and technological conditions. However, future research should incorporate the development of critical material extraction in addition to the current limitations.

The prices associated with green energy technologies, i.e., solar PV and wind turbines, have followed a declining trend for the last decade, where the latest global prices per 1 MW in 2021 reached around 0.8 million USD and 0.25 million USD from wind turbines and solar PVs, respectively[63]. However, the reversal of the cost reduction trend since the beginning of 2021 led to an increase in prices for wind turbines and solar PV modules[64]. This was partially driven by the increase in prices of raw materials, including several critical elements, where the latest prices of REEs, nickel, and aluminum increased 2.5, 3.1, and 1.8 times, respectively, compared to 2017 prices[63]. While the increase in prices in the green energy sector was driven by growth in demand, this might lead to the rebound effect of lower demand in the future. The studied model adopts the projections of increased green energy capacities by the year 2050[10]. However, the price fluctuations and the supply-demand dynamics may create uncertainties in capacity projections. Future research is encouraged to conduct a deeper analysis considering the prices of the various material inputs and the possibility of substitutes for the most expensive elements.

The desired reduction of land used for livestock farming offers new areas for natural vegetation and restoration of biodiversity. However, some land types used originally for grazing, i.e., marginal lands, hold neither agricultural nor industrial value. This work lacks the categorization of land types to draw a clear estimation of the environmental costs and benefits following grassland use reduction. A clear differentiation between the different agricultural lands

used for livestock production is needed to have a clear overview of the environmental consequences of livestock replacement with cellular agriculture.

Most studies on the processes of cellular agriculture production are still on a laboratory scale. The future of scaled-up cellular agriculture production remains vague and holds many uncertainties regarding the wide changes in the inventory inputs and the market situation.

Methods

System dynamics modeling

We applied a global dynamics model of transition to cellular agriculture using a system dynamics (SD) modelling[66] combined with life-cycle assessment (LCA). All inputs of the model, the model structure, and model validity can be found in Supplementary Tables 3, 4, and 2.

The integration of both methods enables examination of the current environmental situation by taking into account various feedback and loops[67]. SimaPro PhD software was used to perform LCA analysis, and Vensim PLE software was used for the system dynamics modelling.

The food system is complex as causal relationships take place externally between socio-economic factors and streams of commodities, and internally between the flows of different commodities (i.e., crops and feed production, livestock production, fertilizer production)[68]. These interactions result in the distinctive behavior of the food system. In addition, the different stages of food production contribute to changes in the energy supply systems and, consequently, material systems[69]. The use of a system dynamics model was motivated by the need to overcome some of the limitations of the model food systems, as model food systems lack the ability to understand the mechanisms behind the consequences or effects of changes in input parameters[70]. Assessing complex systems involving material, energy, food, and environmental consequences requires the consideration of various mechanisms and factors, for example, delay mechanisms, including time lags in various processes and feedback loops; dynamic market factors, including

supply and demand and price fluctuations; geographical-based production; and scenario analysis. System dynamics modelling allows us to understand the structure of the system and to analyze the behavior of variables influenced by several policies over time. System dynamics modelling analyzes multi-systems given their established relationships. The analysis covered a 30-year time horizon (2020–2050). The selection of this time interval is motivated by an intention to explore the changes that cellular agriculture could bring in line with the global roadmap of energy transformation by 2050. The aim of the global roadmap is to limit the rise of global average temperature to well below 2 °C above pre-industrial levels, mainly by carbon sequestration and decarbonization measures, including the deployment of low-carbon technologies based on renewable energy sources[8].

Stocks and flows are the foundations of system dynamics modelling. Stock (i.e., stock of material or energy) corresponds to an entity that accumulates or drains over time, as shown in Eq. 1.

(1)

where is the amount of material accumulated at time; and are calculated using Eq. 2. is the amount of material input to at time; is the amount of material output from at time; and is the amount of material accumulated at the initial time.

(2)

where there is an auxiliary variable (i.e., it is not directly affected by the system components) at time, e.g., the supply flow of secondary fertilizers depends on the organic wastes from food consumption and livestock, and the available stock of organic wastes; is a parameter of the system, e.g., recovery coefficient of wastes to secondary fertilizers.

The dynamics of the system will be driven by the annual global production of food until the year 2050, affected by socio-economic developments leading to the future increase of the demand for crops and livestock[73]. The produced quantities of food commodities were derived from the database of the Food and Agriculture Organization of the United Nations (FAO)[74], while considering the annual growth of population from the United Nations (UN)–population division[75], and the projections by 2050 of per capita consumption from the reports published by FAO for livestock meat, dairy, eggs, cereals, vegetables,

and fruits[76,77]. The global projections in the report applied the categorization of countries into “developing” and “developed” based on gross domestic product (GDP) levels.

System description of the food supply chain

In this study, the global food system was designed following the magnitude of nutrient flows, i.e., phosphorus. Phosphorus plays a fundamental role in the food production–consumption chain where agriculture primarily depends on the inputs of phosphate nutrients[78]. The boundary of the studied system encompassed: (1) extraction, beneficiation, and processing of nutrients; (2) production of fertilizers and chemical feed; (3) production of crops as food and feed commodities (i.e., fruits, vegetables, rice, wheat, maize, barley, rye, sugarcane, and oat); (4) production of cellular agriculture (i.e., MP and cell-cultured RP) and livestock (i.e., beef, lamb, pork, poultry, milk, cheese, and eggs); (5) agriculture waste generation; and (6) downstream processes, i.e., waste disposal, landfilling, and recycling. The analysis of food products followed the cradle-to-gate approach (farmgate for crops and livestock products), excluding the packaging, retail, marketing, preparation, and distribution processes.

The first stage in food production refers to the input of nutrients, i.e., phosphorus. Phosphorus originates from the non-renewable natural resource—phosphate rock—that undergoes several stages of refining following mining activities, beneficiation, and processing. Processed nutrients are next used to produce fertilizers as well as food and feed additives, while the rest is used for non-agricultural purposes such as detergents and various industrial products[18].

Fertilizers—mainly in the form of diammonium and monoammonium phosphates—are applied directly to the soil to enhance the yield and productivity of crops. The uptake of phosphorus nutrients per crop type was derived from Chen and Graedel[79]. Historical statistics show that up to 40% of cereal production is driven by livestock demand and is directly utilized for livestock farming[40]. The remaining crop production is destined for human consumption, although some yield is also used as feed for livestock farming[79].

Pork and poultry have been dominant in the meat production industry, where 37% and 35% of meat originates from pork and poultry species, respectively. Around 21% of meat comes from beef and cattle, while the rest comes from sheep, goat, horse, camel, and duck[80]. In the dairy industry, cattle and buffalo contribute to >95% of global milk production, while the rest originates from goat, sheep, and camel[81]. Around 30% of the globally produced milk is used for cheese production based on protein content[74], by considering 3.5% and 25% of protein content in cow milk and cheese, respectively[82,83]. The data on phosphorus contents in meat varieties, dairy, and egg products were obtained from the U.S. Department of Agriculture (USDA)[56]. For poultry production, chicken meat was used as it accounts for more than 90% of total poultry meat globally[84].

After food production from livestock and crop origins, ~30% of products ready for consumption are wasted on an annual basis[85]. Concerning livestock production, 10% goes to the consumption stage, while the rest ends up as waste, 90% of which is used as organic fertilizers for direct application on croplands in the form of animal manure[79]. Besides the flows of manure, recycled nutrients from wastewater are also used as organic fertilizers[86,87].

Nutrient losses occur throughout the food supply chain. Mining, beneficiating, and processing of raw natural resources of phosphorus lead to 28% losses[88]. During agricultural production, runoff of nutrients from the soil is the main reason for phosphorus losses to marine ecosystems (around 28% of total nutrient input to the soil)[79]. This runoff phenomenon is driven by the leaching of excess nutrients that are not absorbed by crop plants, leading to the accumulation of phosphorus in water[89]. Contamination of water surfaces arises from animal waste, feeding operations, food consumption, crop harvesting, and wastewater treatment processes[79,90].

Cellular agriculture production

The production of cellular agriculture was based on the protein content of livestock products. The protein content for chicken broiler, beef, pork, lamb, egg, cow milk, and cheese was derived from the USDA[56].

In this study, phosphorus inputs were obtained from the production of phosphoric acid after beneficiating and processing primary material from extracted phosphate rock. Approximately 20% of phosphorus input to MP and cell-cultured RP was released to wastewater streams, following the calculations by Järviö et al.[29], with regard to the wastewater generation phase after the fermentation during the MP production process. The recovery processes for wastewater were included in the production system. By extension, the calculations of the environmental indicators (i.e., energy demand and GHG emissions) encompassed the recovery processes.

Mathematical model of food production

In the model, the world production of crop products is calculated as the production of crops per capita multiplied by the population as follows (Eq. 3):

(3)

where is the world population at time ; (t) is the annual crop production per capita at time. Livestock production depends on livestock demand and the replacement coefficient with cellular agriculture products. In this step, livestock demand was calculated based on protein content, as the cellular agriculture transition was based on protein replacement. The protein contents of the livestock products were derived from the USDA[56]. Equation 4 corresponds to livestock protein demand at time.

(4)

What is the demand for livestock product protein (beef, lamb, pig meat, poultry, milk, cheese, and eggs) per capita at a time? The production of cellular agriculture depended directly on the livestock protein demand and the replacement coefficient at the time. For livestock meat proteins, MP and cell-cultured RP proteins are produced to totally replace livestock protein products (Eq. 5):

(5)

Cell-cultured RP is produced to replace only dairy and egg products (Eq. 6):

(6)

Description of the energy consumption system

The World Food LCA database[37] and Agri-footprint 5.0[38] were utilized to calculate the energy requirements for the production of crops. (Details on the inventory data and energy values can be found in Supplementary Method 3, Supplementary Tables 1 and 4). The total energy demand covered the cradle-to-farmgate processes. Only crops meant for human consumption were included in this category, while the energy needed for feed crops was included in the livestock production sector. The energy required for the livestock meat, milk, and eggs production was derived from Vries and Boer[92], where 16 peer-reviewed works on the environmental impact of livestock production were analyzed by cradle to farmgate LCA. Studies encompassed OECD countries, as the use of livestock animals was driven by the demand for livestock food products. The average consumption of energy was determined and used in our model. The energy requirements for cheese to the farmgate were derived from Finnegan et al.[93]. The study reviewed five assessments of the cumulative energy demand of cheese production in the Netherlands, USA, Spain, and Portugal.

The energy requirements for cellular agriculture were derived from Järvio et al.[29,30]—53 kWh per kg MP and 44 kWh per kg cell-cultured RP proteins. Further information on the total energy demand of cellular proteins can be found in Supplementary Fig. 2.

The energy requirements for nutrient recovery from wastewater were derived from Spångberg et al.[94]. Energy consumption is associated with two subsequent methods, 'thermo-chemical treatment' and 'wet chemical approach'[95]. The 'thermo-chemical treatment' is meant to remove heavy metals from sludge phases via the addition of chloride additives. This facilitates the extraction of phosphorus from those sludge phases via the 'wet chemical approach' by adding strong acids[86].

The general mathematical formulation of energy consumption in the food system is a follows (Eq. 7):

(7)

where is the energy demand per tonne product and is the production of product at time.

Greenhouse gas (GHG) emissions

The assessment of GHG emissions considered the carbon equivalent emissions from primary production of nutrients, fertilizers, crops, livestock meat, dairy, and eggs, recycling of nutrients, and production of cellular agriculture products.

The study by Poore and Nemecek[36] was taken as a reference for the carbon footprint per tonne of livestock and crops. The global mean data of the analyzed studies were used for the global assessment. The percentage accuracy of the obtained results can be found in Supplementary Method 5. The system boundary of the study was up to the retail stage. We excluded the emissions in post-farmgate stages, which constituted around 17% of total GHG emissions from the food system. The GHG emissions associated with agricultural products at the time are calculated as follows (Eq. 8):

(8)

where is the intensity of GHG emissions per tonne product (Supplementary Table 4); and is the production of product at time.

The GHG emissions associated with cellular agriculture at time are calculated with Eq. 9 as follows:

(9)

where is the intensity of GHG emissions per tonne of cellular agriculture proteins (MP and cell-cultured RP); and is the amount of the production of cellular agriculture protein at time.

For the recycling stage of nutrients, GHG emissions are estimated based on the energy consumed during recovery operation using three streams: wastewater, manure, and solid wastes. The global distribution of utilized energy sources was adopted to allocate the energy mix for the recycling operations. The latest results showed the following distribution of global energy consumption according to the energy source: petroleum oil (31%), natural gas (24%), coal (27%), hydroelectric (7%), nuclear (4%), and wind and solar PV (7%)[35].

The calculation of generated GHG emissions from nutrient recycling at a time is calculated using Eq. 10.

(10)

where is the energy consumption in the recycling process at time from the energy source; and is the intensity of GHG emissions per one kilowatt hour of energy consumed from the source. GHG emissions from different energy sources can be found in Supplementary Table 4.

Agricultural land use

In this study, agricultural land consisted of land for the production of crops (arable land) and land for livestock grazing (pasture and grassland). Poore and Nemecek[36] were used as a reference, as they provided detailed results on the agricultural land demands per unit of food product (Supplementary Table 4). Land use for livestock production refers mainly to the cultivation of feed crops and the grazing activities of animals. Hence, the land use for livestock meat also included the land requirements for feed crop cultivation on arable lands. The total land use for livestock production at time is calculated by Eq. 11 as follows:

(11)

Where is the land use per tonne of livestock product, and is the production of the product at the time? The cropland area used for the production of crops for human consumption refers to the land requirements for the production of embodied nutrients in crops (i.e., P). Hence, cropland area used for food production at the time is calculated using Eq. 12 as follows:

(12)

Where is the land area to produce one tonne of crop for food, and the amount crops are produced at a time? The total arable land used for livestock production was derived from Poore and Nemecek[36], constituting a coefficient of around 0.16 of total land requirements for livestock products. The coefficient reflects the share of arable land used for the production of all livestock species. It was assumed to be constant as the shares of meat production from different animal species are not subject to change until 2050[76]. Equation 13 corresponds to land use for feed crop production, and Eq. 14 corresponds to pasture land use at the time.

(13)

(14)

The land use for cellular agriculture was derived from Järviö et al.'s publications on MP[29] and cell-cultured RP[30], given that the production is

powered by green energy technologies (solar PV and wind). The arable land used in the cellular agriculture production is limited to the production of maize as a glucose source for cell-cultured RP, and is calculated as follows (Eq. 15):

(15)

Where are the land use requirements per tonne of cell-cultured RP protein, and what is the amount of cell-cultured RP protein at a time?
Equation 16 gives the arable land use as follows:

(16)

Demand for infrastructure—green energy and stainless steel

The demand for green energy and stainless-steel structures depended on factors including the average lifetime of the structure and requirements per resource flow. The studied model encompasses several technologies across the food system (mining, production, and recovery technologies), cellular agriculture system (microbial protein and cell-cultured recombinant technologies), and energy supply system (wind and PV solar technologies). Researchers have estimated that the development of green energy supply technologies showed changes in material intensities until 2050, mainly driven by the need for higher efficiency in production processes[10]. The amounts of materials required for the construction of wind turbines (i.e., zinc, nickel, chromium, aluminum, and manganese) showed a decreasing trend, while the amount of materials required for the function of wind turbines and solar PV panels showed an increasing trend (i.e., tellurium, germanium, gallium, indium, rare earth elements, and boron)[10]. It should be noted that the ratios could be adopted in the model based on a newly identified technology or energy source.

Equation 17 corresponds to the total amounts of unit (i.e., solar PV panel, wind turbine, and tonne of stainless steel) required for the food system.

(17)

where Eq. 18:

(18)

and Eq. 19:

(19)

where is the amount of units needed at time, and is the average lifetime of a unit. For green energy structures, was calculated using Eq. 20:

(20)

where is the amount of units required per 1 kWh of electricity (wind turbines, solar PV panels); and is the electricity generated from wind or solar energy sources for the food system at time. For stainless-steel structures, it was calculated as follows (Eq. 21):

(21)

where is the amount of unit (tonne stainless-steel) per tonne material flow at the sector (dairy products, cellular agriculture products, and nutrients for wastewater treatment); and is the mass flow of material in the sector at a time. Data on equipment requirements can be found in Supplementary Table 4.

Demand for critical materials in the food system

The material intensities for green energy technologies were derived from the European Commission publication on the materials demand for wind and solar PV technologies[10]. The equation below is used to calculate the critical material demand at time (Eq. 22):

(22)

where is the critical material content at time per one unit (solar PV panel and wind turbine) (material intensity in solar panels and turbines can be found in Supplementary Table 4); For stainless-steel, 304 and 316 stainless-steel grades had no marked difference in using the critical material composition (1% difference for chromium and nickel). Hence, the average of the material composition in each grade was taken for the model.

Country-level cellular agriculture production

The calculation of the cellular agriculture protein to replace livestock production at a country-level was based on the associated availability of wind and solar PV capacities, as the direct cellular production was aimed to be fueled by green energy technologies only.

First, the country-level green energy capacities in 2050 are derived from Ram et al.[96]. Second, the country contribution to the world livestock protein production is calculated based on the latest country-level and world livestock production from the FAO database[74]. Third, the country-level livestock protein production by 2050 was calculated by multiplying the country's contribution to the projected global production in 2050. Country-level data on livestock production and green energy capacities can be found in Supplementary Data 1 and 3, respectively. Fourth, the direct electricity needed for the country replacement with MP and cell-cultured RP in 2050 was calculated using Eq. 23 as follows:

(23)

where is the estimated amount of cellular agriculture proteins, in tonnes, used to totally replace livestock protein products in the country; is the direct electricity needed to produce one-tonne cellular agriculture product = 1, 2 (MP and cell-cultured RP) in protein content, respectively. Considering the limited national capacities of wind and solar PV, the electricity consumption that a country would be able to handle to replace livestock proteins with cellular agriculture is calculated as follows (Eq. 24):

(24)

Where is the electricity originating from wind energy sources, and is the electricity originating from solar PV energy sources, which are calculated with Eqs. 25 and 26 as follows:

(25)

When wind power technology is given priority, and

(26)

when solar PV power technology is given priority. and denote the regional wind and solar PV energy capacities in the country, respectively. The total livestock protein products to be possibly replaced by the country with cellular agriculture in 2050 was calculated as follows (Eq. 27):

(27)

Primary production capacities of critical materials

The primary production capacity of several materials was derived from the latest publications of USGS, including aluminum[97], chromium[98], indium[53], rare

earth elements[99], gallium[100], nickel[101], and tellurium[49]. The Statista database was used for the primary production capacity of phosphorus[102]. While for other materials, data was collected from regional reports for major world primary producers, i.e., germanium[103], boron[104], manganese[105,106], zinc[107,108,109,110,111,112], and silicon[51,113]. Detailed information on the aggregation of data presented (Table 1) can be found in Supplementary Method 4.

Table 1 Primary production capacity of studied critical materials.

It is important to note that technological advancements and resource discoveries can also increase production capacities in certain cases. Additionally, the likelihood of capacity decrease varies for different materials and industries, as each has its own unique dynamics and influences.

Alternative proteins will transform food, mitigate climate change, and drive profits. Here's how:

- **Plant-based, microorganism- and animal cell-based alternatives to animal meat, fish, eggs, and dairy are projected to make up at least 11% of global protein consumption in 2035 – with a push from regulators and step changes in technology, they could reach fully 22% of the total.**

- **By 2035, the shift to plant-based meat and eggs alone would save as many carbon emissions as Japan emits in a year, enough water to supply the city of London for 40 years, and promote biodiversity and food security.**

- **A new report from Blue Horizon (BHC) and Boston Consulting Group (BCG) says the $290 billion alternative protein market is the foundation of a more sustainable food system.**

The world's consumers love animal-based protein—so much that in 2020 they ate 574 million metric tons' worth of meat, fish, dairy, and eggs—almost 75 kilograms per person. And the amount consumed is growing, especially in developing economies. Yet concerns about the environmental costs of raising all the animals we eat, how

those animals are treated, and the consequences for human health of eating all that meat, fish, dairy, and eggs are growing even faster.

That's why alternative proteins have morphed in just the past few years from a niche product to a mainstream phenomenon. Plant-based meats are now a fixture at fast-food restaurants around the world, and plant-based milk is a household staple. Microorganism-based alternatives have been available for decades, and you can taste meat grown from animal cells in restaurants in Singapore and Israel.

Indeed, nine out of ten of the world's most popular dishes will soon be feasible with reasonably priced alternative proteins, especially those using less-structured meat, such as ground beef.

We expect the alternative-protein market will increase to more than seven times its current size over the next decade and a half, from a current 13 million metric tons a year to 97 million metric tons by 2035, when it will make up 11% of the overall protein market. Assuming average revenues of $3 per kilogramme, this amounts to a market of approximately $290 billion.

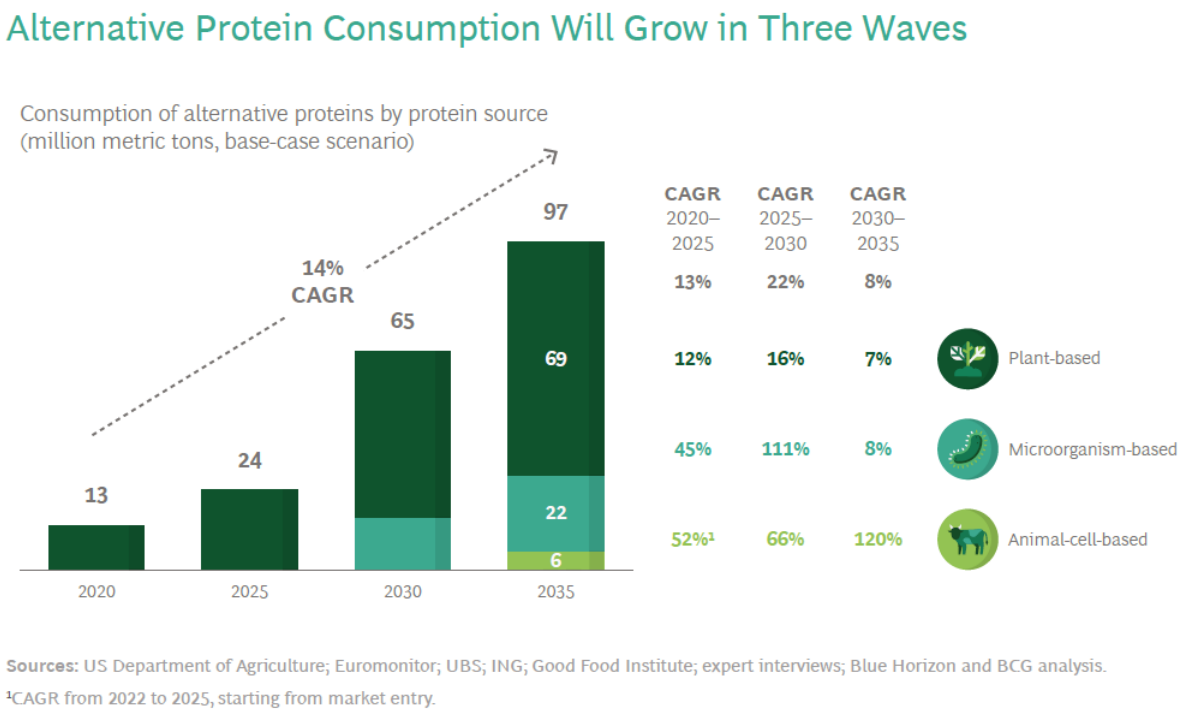


Image: Image: BHC and BCG

These figures assume a consistent pattern of consumer acceptance, regulatory support, and technological change. How would changes in these assumptions affect the

market's growth? Significantly, and mostly on the upside, according to the alternative scenarios we developed.

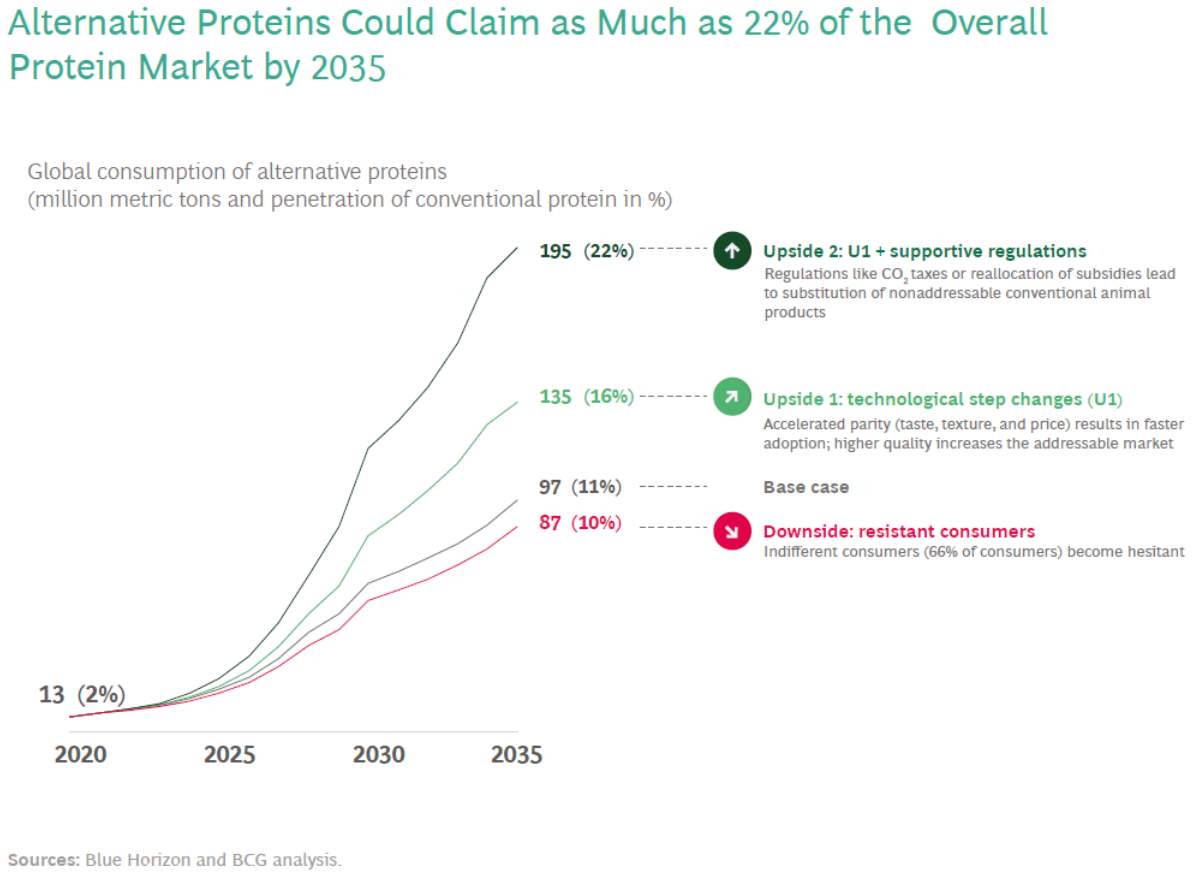


Image: Image: BHC and BCG

- **Upside #1**. Further efforts on the part of scientists, startups, incumbents, and investors lead to technological step changes that improve product quality and accelerate the time to parity. Alternative proteins grow more quickly, to 16% of the market in 2035.
- **Upside #2**. More supportive policies and regulations, such as widespread taxation of greenhouse gas emissions or reallocation of agricultural subsidies to support the transition to alternative proteins, make alternative proteins substantially less expensive than conventional animal foods, pushing penetration to 22% of the market in 2035.
- **Downside**. Consumers who currently say they are ambivalent about protein substitutes become more hesitant to try them, slowing market growth to 10% of the 2035 total.

Already at 11% of all protein, the protein transformation offers substantial benefits. With the shift to plant-based meat and eggs alone, the world could already save one billion tons of carbon emissions from now until 2035 – equivalent to Japan going carbon neutral tomorrow, for an entire year.

On top come 39 billion cubic meters of water, which could supply the city of London for 40 years, and an area the size of the United Kingdom that would not be needed for agriculture. The number of animals undergoing ethically questionable treatment in factory farms can be reduced – for instance, 50 billion fewer chickens would be eaten from 2020 to 2035.

Biodiversity, the climate, and human health would receive a much-needed boost, in line with six of the UN Sustainable Development Goals. For the individual consumer, this means you can save as many carbon emissions as a new car emits on a 10km ride, just by making one portion of spaghetti Bolognese with the plant-based mince you can already buy today in most parts of the world.

Whether or not any of these alternative scenarios comes to pass, substantial capital must be invested across the protein value chain to support the protein transformation. The extrusion capacity needed for plant-based proteins, for example, will require up to $11 billion to reach the baseline case of 11% adoption by 2035, and as much as $28 billion if the greatest upside scenario comes to pass.

Almost 30 million tons of bioreactor capacity for microorganisms and animal cells will also be needed in the base case, requiring up to $30 billion in investment capital—and far more, in either of the upside scenarios. These are just two of the technologies required to unlock the market's growth; the total capital needed will likely be much higher.

Most investment capital is currently focused on companies that are integrated along the value chain, enabling them to ensure quality control as they explore new technologies. As the industry matures, however, two types of investment plays will emerge.

The first is a technology play: a company that solves a particular technological challenge will likely become the go-to firm for its specific step along the value chain, such as flavoring, and other companies will eagerly license its intellectual property to augment their processes. The second is a platform play: well-funded companies or investors will build industrialized platforms for capital-intensive technologies such as extrusion.

A tasty, sustainable food system

The benefits of alternative proteins are clear: tasty, nutritious, and healthy food, lower carbon emissions, and fewer concerns about the ethics and environmental consequences of intensive animal farming.

Farmers and scientists are at the heart of the transformation, providing the technological means and the quality inputs needed. Incumbent producers and startups will refine and scale up production to make alternatives tastier and less expensive, securing market shares in the race to parity.

Consumers will demand more enjoyable alternative proteins. Investors with the right vision and expertise can fund the transformation and participate in every step of the value chain. Together, they can benefit from a $290 billion market as they build a more sustainable food system that tastes good, too.

See the joint Blue Horizon and BCG report "Food for Thought: The Protein Transformation" for more insights.

Fermentation helps build a more sustainable food system – here's how:

Fermentation presents an opportunity to fundamentally change how the world eats and improve global human and environmental health and the economy.

Natalia Suescun Pozas
Partner Lead, Latin America, World Economic Forum
Caroline Bushnell

Our global food system is not fit for purpose. Current food production methods and consumption patterns endanger the health of the public and of the planet.

But demand for food and protein is only going to grow. By 2050, projected demand for protein is set to nearly double globally as incomes rise and the population reaches an estimated 10 billion.

According to the World Economic Forum, using today's production systems, we will not be able to match the future demand for protein and meet the targets of the Sustainable Development Goals and Paris Accord. To create a global food system that is inclusive, efficient, and sustainable, we must transform protein production methods.

Industrial animal agriculture is inherently resource-intensive, exacerbating some of the world's most serious problems, including land-use conversion, environmental degradation, and climate change. The UN's Food and Agriculture Organization (FAO) determined that total emissions from global livestock today represent 14.5% of all anthropogenic greenhouse gas emissions. Industrial animal agriculture also poses significant public health concerns with the potential to drive antibiotic resistance and foster conditions ripe for the spread of zoonotic diseases like COVID-19.

Enter alternative proteins, which not only have the ability to vastly improve our food system, mitigate the impact of food production on climate change, and greatly improve public health, but also hold tremendous opportunities for innovation, investment, and economic growth.

What is alternative protein?

The alternative protein field has three main pillars: plant-based protein, cultivated meat, and fermentation.

Plant-based protein sales are rapidly growing, outstripping the growth of conventional meat even during COVID-19.

Cultivated meat ("clean meat" or cell-based meat) is the darling of futurists – and quickly becoming a reality. By successfully growing cells outside of an

animal, multiple companies have offered proof-of-concept tastings ranging from steak to shrimp. Together, two of the leading startups in the field – Memphis Meats and Mosa Meats – raised over $240 million this year in Series B financing, revealing investors' confidence in cultivated meat's potential to capture a meaningful share of the $1 trillion-plus global meat market.

Until recently, the third pillar hasn't received much attention. However, **fermentation**– an ancient production method now transforming our modern food system – is starting to receive the attention it deserves.

Why fermentation is the next frontier of alternative protein

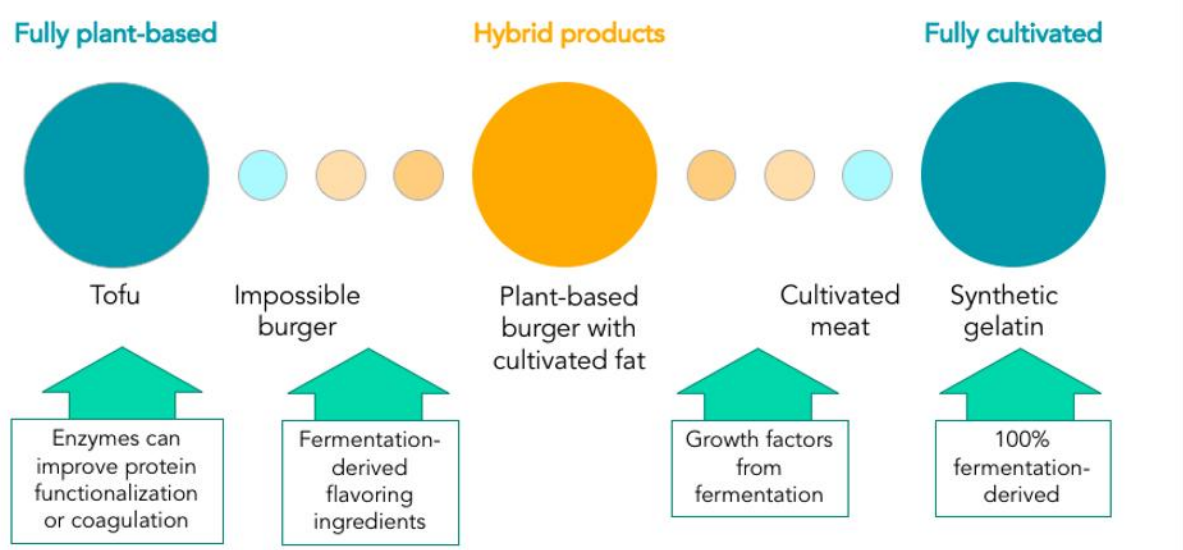
Within fermentation, there are three primary production methods of alternative proteins:

Microbial fermentation has been used for many years to produce ingredients such as enzymes, where microorganisms transform one type of food into another (think beer or yogurt), and in the biopharma industry to produce vaccines and drugs. The same technology is now being applied to produce a new generation of proteins, fats, and other functional ingredients that enable the creation of animal-free meat, eggs, and dairy.

Many may recognize **whole-biomass fermentation** from Quorn's meat-free mycoprotein nuggets, patties, and fillets. There are also innovative startups working to produce whole-muscle products using whole-biomass fermentation, such as Atlast Food Co. and Meati.

The third method, **precision fermentation**, uses customized microbes to produce vast quantities of specific proteins normally found in animal products but without having to breed, feed, and slaughter an animal. The most well-known example is rennet, a protein found in nearly all cheese. Rennet traditionally comes from the stomachs of slaughtered calves, but now can be produced by a special strain of yeast. Perfect Day Foods is making whey and casein proteins using precision fermentation and recently launched a spinoff brand, Brave Robot, to sell animal-free dairy-based ice cream. Clara Foods is also creating egg proteins with this technology.

Each fermentation method has the power to make protein production more efficient. More broadly, the pillars of the alternative protein industry – plant-based, cultivated, and fermentation – can complement each other, allowing companies to create more environmentally sustainable and less resource-intensive products than those that currently come from industrial animal agriculture.



Types of alternative proteins made by fermentation

For example, Impossible Foods' "heme," the ingredient that gives their burger its "meaty" taste, uses a microbial fermentation process similar to rennet production. Such products can be used along the entire alternative protein spectrum, making them a critical component of the industry.

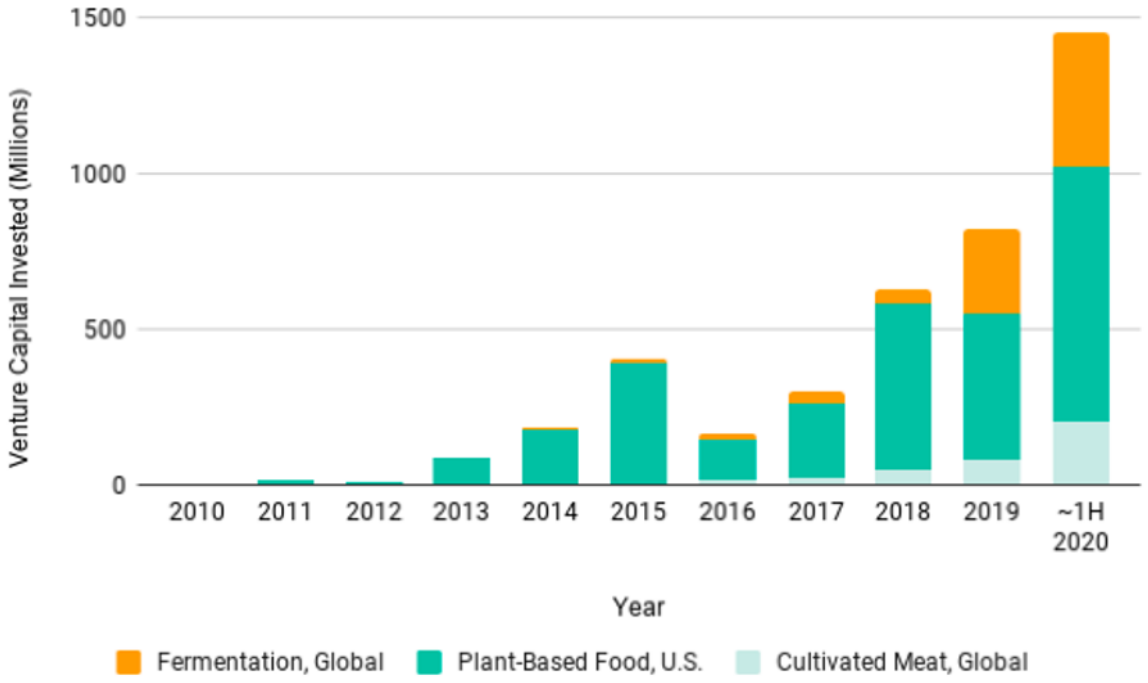
Another benefit of fermented products is that, like plant-based proteins and cultivated meat, they can be produced efficiently, sustainably, and without the use of antibiotics. By taking the animal out of the system, the constant threat of contamination is removed, and the need for antibiotics in our food system is eliminated. This would also eliminate the threat of cross-species disease transfers. All of this is a tremendous boon to public health and the security of our food (and thus economic) system.

An investment opportunity

Venture capital investment across the alternative protein industry has grown rapidly over the past decade, with significant acceleration over the past few years. In the first half of 2020 alone, alternative protein funding substantially surpassed the amount that the industry raised in the entirety of 2019, eclipsing $1 billion for the first time. And fermentation is claiming an increasingly large

share of that amount. The momentum is incredible, with 85% of all VC investments in alternative protein fermentation companies having occurred in just the last 18 months.

Seeing the opportunity, several of the largest food and life science companies in the world – including DSM, DuPont, and Novozymes – are developing fermentation-based product lines and solutions tailored to the alternative protein industry. JBS, the world's largest meat company, is leveraging fermentation for alternative proteins in its Ozo plant-based products.



Investors are taking notice of the opportunity of alternative protein.

Despite this growth, much more investment is needed.

Fermentation presents an opportunity to fundamentally change the way the world eats. Companies have developed promising fermentation technology, but they are nascent, under-resourced, typically Western-based, and few. While fermentation itself is a relatively mature platform, significant opportunities for innovation and investment exist at every step of the process: from ingredients, to feedstock, to production facilities, to flavor and texture improvements.

For example, the feedstock – or nutrient source – used for fermentation has enormous implications for both cost and sustainability, as well as affecting the growth, composition, and flavor of the microbes themselves. While fermentation processes have historically relied primarily on purified sugars as feedstocks, there is huge potential to leverage lower-cost inputs and simultaneously process side streams from other industries to eliminate waste.

Additionally, the largest fermentation facilities in the world were built for other industries rather than for alternative protein applications. And the largest facilities for alternative protein fermentation are orders of magnitude smaller than animal meat processing facilities. Thus, there is immense room for capital influx to scale up manufacturing capacity and infrastructure.

In 2019, economic think tank RethinkX released a report predicting the potential disruption and collapse of the industrial livestock sector. However, this prediction depends heavily on diminishing costs and increasing quality of precision fermentation.

For fermentation-derived products to reach price parity with animal-derived versions, it's critical to drive innovation and investment towards achieving the necessary scale and technology. In doing so, fermentation presents a clear opportunity for investors to both revolutionize modern protein production and help build a healthier, more efficient, sustainable global food system.

How Big Food is responding to the alternative protein boom

On average, humans slaughter over 70 billion animals for food every year. That's 130,000 animals every minute.

This scale is only possible because we've transformed animal agriculture from a predominantly pastoral, family-owned farming system to an industrialized system of livestock production. It's one that has created a $1.5 trillion industry, serving as the backbone for the rise of Big Food and inspiring iconic products, from the Big Mac to the Whopper. It has made animal proteins – whether from milk, meat, or fish – so ubiquitous that the price of a Big Mac provides a standard to gauge currency misalignment between countries, and chicken bones may serve as an index fossil that defines the Anthropocene.

But increasingly, it's a system that is under scrutiny for the scale of its impact on people and the planet. These concerns are driving consumers to seek substitutes for the meat and dairy they love and inspiring innovators to meet this market demand through disruption: replicating the taste, texture, and flavor of animal proteins with better resource efficiency and without the actual animal.

These developments have challenged the long-held thesis that the only way for the sector to grow (and feed a world of 10 billion people by 2050) is through the expansion of our current intensive animal production system. For the first time since the advent of industrial animal agriculture nearly 60 years ago, alternative proteins – whether plant-based or cell-based – present a viable path forward to meet global demand for proteins sustainably.

Since 2016, FAIRR – a network of over 250 institutional investors – has coordinated the world's only collaborative investor engagement with 25 global, listed food companies. Our engagement encourages food giants to adopt a strategic approach to diversifying protein sources away from an over-dependence on animal proteins – both to leverage market opportunity and to de-risk supply chains.

Here are six essential takeaways from FAIRR's new report, *Appetite for Disruption*, on how Big Food is responding to the alternative protein boom:

1. Protein diversification is a driver of business growth

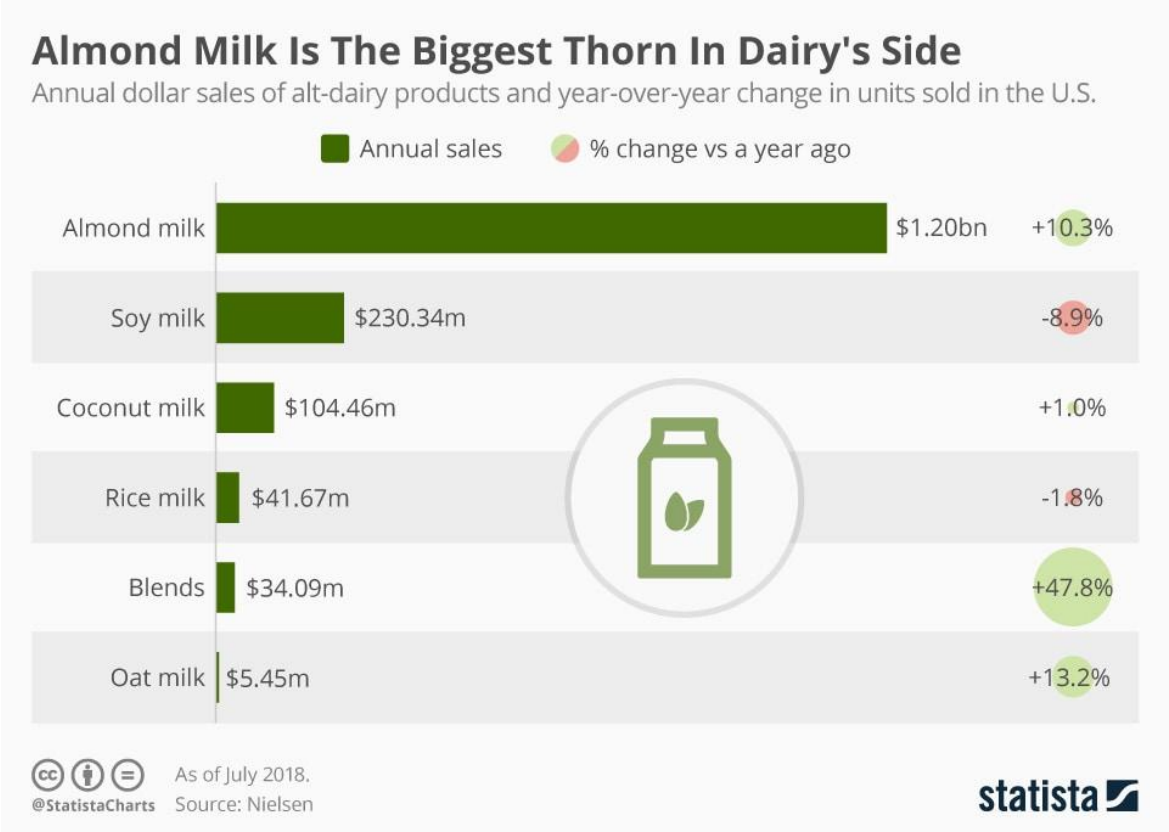
Plant-based foods are leading growth for the food sector: in the US, sales of plant-based foods have grown by 31% over the last two years vs 4% for retail food sales. It's no wonder that terms like "plant-based" and "vegan" now regularly appear in company communications: in 2018-19, 64% of the companies in FAIRR's engagement referred to these terms in their annual reporting and quarterly earnings calls to investors.

2. Alternative proteins are a disruptive force for the food industry

The rapid rise of the plant-based market has producers, retailers, and food brands playing catch-up. New entrants like Beyond Meat, Just, and Impossible Foods have forced established players to innovate, invest, and launch new products to avoid the disruption of their existing brands.

Just this year, analysts from JP Morgan, Barclays, AT Kearney, and UBS have weighed in on how quickly alternative meat will capture market share

from traditional meat. Milk alternatives have already grabbed market share and now account for 13% of US retail milk sales.



3. Supply chain interventions are not enough on their own

No company has yet successfully demonstrated a measurable reduction in risks associated with animal protein supply chains. Many have not even begun the journey: only 28% of companies in FAIRR's engagement have set targets to reduce supply chain emissions. Despite years of working to curb deforestation from soy (predominantly used for animal feed) and cattle-ranching, most food companies will not be able to meet their target to halt deforestation by 2020.

This drives home a clear message: a sole focus on supply chains to tackle risks will require increased capital investment with limited benefits as demand for meat, dairy, and fish grows. These initiatives will have to be balanced against growing consumer demand for better welfare, lower antibiotics use, slower-growing breeds, no confinement, and no routine mutilation, all of which will result in higher costs. This will ultimately put pressure on an industry where net margins are small to begin with, averaging around 2-3%.

On the other hand, reducing exposure to resource-intensive commodities can have immediate benefits – in 2019, General Mills decreased emissions from agriculture by 17% versus its 2010 baseline, primarily due to reduced purchases of dairy.

4. Global food companies are rapidly expanding their alternative protein portfolios

Our analysis identified that all 16 retailers have expanded their alternative protein product portfolios in the last year.

For retailers, private-label ranges present an important opportunity to expand their selection of affordable alternative protein options. In the US, for example, sales of private-label brands grew at 4.3%, vs. 1.2% for external brands. Within our engagement, over 87% of retailers, apart from Costco and Walmart, have ramped up their private label alternative protein products across categories to compete with external brands.

On the manufacturing side, companies are expanding their exposure to alternative proteins primarily through acquisitions, venture investments, and new product launches. Making early-stage minority investments into start-ups is a popular approach. It gives corporates access to innovation across a broad range of technologies and to evolving consumer trends, as an alternative to making a larger commitment to strategic acquisition (the more traditional M&A route). Eight of the nine manufacturers in our engagement now demonstrate a combination of all three approaches.

5. Alternative proteins have kick-started a new era of regulatory and lobbying activity

Overall, regulatory activity is favourable to the sector: both the EU and the US have updated their regulatory processes to cover novel ingredients and cell-cultured products, reducing the barriers to market entry for alternative proteins. In the US, the pathway to regulate cell-cultured meat was especially contentious after an intense inter-agency dispute on who should regulate the industry. In 2018, the US Department of Agriculture (USDA) and the FDA agreed to a joint regulatory framework. However, regulation is also being used as the new battleground by the traditional (and powerful) animal protein lobbies to push back on the alternative protein sector's explosive growth. In the US, 24 states have passed legislation barring the use of words like "steak", "burger", and "milk" for plant-based foods.

In response, the alternative proteins industry is organizing to protect and advocate for its interests. Organizations like Plant Based Foods Association and the Good Food Institute in the US and the European Plant-Based Foods

Association in Europe are working to advance the policy interests of the sector. Big Food is now entering the space: both Kellogg's and Campbell Soup are now part of the Plant-Based Foods Association. Many alternative protein start-ups are also working with longstanding food industry veterans to help them navigate regulatory complexities and build and scale their go-to-market strategies.

6. Lessons for a shifting protein landscape

Our methodology measures how prepared Big Food is when it comes to leveraging alternative proteins for growth and risk mitigation. We assessed 25 companies on their recognition of risks linked to their animal protein supply chains (materiality); their work to tackle risks through commitments (strategy); how they are expanding alternative protein portfolios (product expansion & consumer engagement); and finally, whether companies are measuring their progress (tracking & reporting).

Our findings indicate that Unilever demonstrates the most proactive approach to protein diversification. The company recognizes the materiality of the issue for its business, is working to mitigate these risks through its supply chain interventions and science-based climate target, and has been expanding its access to alternative proteins through acquisitions, product development, and clever consumer engagement.

On the other hand, we found Costco's approach to be the most reactive. Despite being one of the largest sellers of chicken and beef in the US, Costco has limited discussion of the impacts of these supply chains and is increasing its exposure to these commodities. While the company has expanded its range of plant-based external brands, we found limited innovation in its signature private-label brand, Kirkland.

For companies and investors, an expanding alternative protein portfolio is a clear lever for growth. More importantly, it is a fundamental and necessary component to manage a company's environmental and social risks while improving its ability to compete and innovate.

As FAIRR's founder, Jeremy Coller, has said: "A fourth agricultural revolution is underway in the world of protein production." The billion-dollar question is, will Big Food keep pace?

Why the future of food must also be blue as well as green

Fish is food. We know that. And yet, in discussions about the future of food, that simple fact tends to be forgotten. When world leaders gather for the UN Food System Summit next year, fish and other aquatic foods need to be on the table.

We confront a daunting challenge. How do we provide a healthy diet to 10 billion people in a way that the Earth can sustain? Deliberations on this question tend to quickly center on questions of livestock, row crops, and the marvels of biotech. Yet our current fixation on land-based food production has come at enormous cost. Agriculture is the principal driver of deforestation and biodiversity loss around the world and is responsible for around 30% of greenhouse gas emissions.

The environmental footprints of aquatic foods ('blue foods') can vary significantly. Some fisheries and farms are responsible for significant greenhouse gas emissions or ecological impacts. But many aquatic foods outcompete terrestrial livestock in terms of environmental impacts. One reason is that compared to terrestrial animals, fish do not need to waste energy on maintaining body temperature or combating gravity and can invest more of their metabolic energy into growth.

Blue foods are already a principal source of protein for some 3 billion people. In many of the most vulnerable countries, they are also an essential source of vital micronutrients, such as iron and zinc, and constitute a key preventive measure against premature deaths and stunting.

Given their relative health benefits, comparatively small environmental footprint, and the potential for a substantial expansion of global production, foods from ocean and freshwater systems have a key role to play in achieving both a healthy and sustainable food system. Yet many discussions of food neglect blue foods altogether. And when they do include blue foods, they tend to deal with them in sweeping generalizations – typically calling for massive expansion of aquaculture.

When we ignore this important food group, we make bad decisions. Terrestrial and aquatic food systems are deeply interconnected, which means that if we don’t think of them together, as one integrated system, we create tradeoffs we regret. For example, intensification of fertilizer use in agriculture has created massive dead zones in coastal waters, with devastating impacts on aquaculture and the nursery areas that support capturing fisheries. Demand for fishmeal to feed livestock has driven massive extraction of small pelagic fish, decimating local fisheries that are often the most important source of nutrition for vulnerable communities. When decision-makers ignore blue foods and view aquatic environments merely as providing inputs to, or absorbing the waste from, terrestrial production, they incur significant costs and miss important opportunities.

As we bring blue foods into the food picture, we also need to recognize that they are stunningly diverse. More than 1,800 species are harvested for food, they are produced in many different ways, and they offer a wide range of benefits to coastal communities and nations. Blue food species vary widely in the nutrition they provide and the roles they play in diets. Small pelagic fish, like sardines, for example, are often consumed whole and provide a rich list of essential micronutrients and healthy fats, particularly important in poor communities with limited access to well-balanced diets.

Blue foods also vary in their environmental footprint, depending on how they're produced, processed, sold, and consumed. Fishers that operate locally may have much lower greenhouse gas emissions than factory trawlers that drag heavy nets across the ocean floor and stay out at sea for months at a time. Clams and bivalves that feed off nutrients in the water have a much lower footprint than shrimp and salmon that require large amounts of protein in feed (and, in intensive cultivation, also require inputs of pesticides and antibiotics). Even fish that are caught or raised close to market can pick up a heavy carbon footprint when they are shipped across the ocean for processing and then shipped back for sale.

Blue foods will not be a silver bullet for fixing the global food system – but they are a piece of the puzzle we cannot afford to ignore. As we bring them fully into debates and decision-making about the future of food, we must be thoughtful about these details – about the interconnections that shape tradeoffs, and about the diversity that presents both challenges to policy and markets and also great opportunities for prosperity and resilience.

What is the World Economic Forum doing to help ensure global food security?

The Blue Food Assessment, convened by Stanford and the Stockholm Resilience Center, has set out to address this need. A consortium of leading researchers from all over the world are producing a set of multi-disciplinary scientific analyses that will elucidate the roles blue foods can play in creating a healthy and sustainable food system and the challenges that must be addressed. The Assessment will lay the foundation for a better understanding of blue foods and integrating them into food system decisions.

United Nations Secretary-General Antonio Guterres is convening the Food Systems Summit to spur the bold actions required to address hunger and diet-

related disease and to heal the planet. An ambitious preparatory process has set out to mobilize action on nutrition and livelihoods, on sustainable production and consumption. To succeed, it must recognize and embrace the roles of food from the water.

CELLULAR FISH AGRICULTURE

Cellular fish agriculture, also known as cellular aquaculture, is an innovative approach to seafood production that utilizes cell cultures from fish and shellfish to create nutritionally complete seafood products. This method offers a sustainable, climate-resilient, and ethical alternative to conventional fishing and fish farming. The process involves isolating embryonic stem cells, adult stem cells, or induced pluripotent cells from the species of interest, which are then cultivated in bioreactors to produce seafood products. This approach not only addresses the growing demand for seafood but also aims to reduce ecological footprints, mitigate overexploitation of marine resources, and improve animal welfare.

Cellular fish agriculture represents a transformative step towards a more environmentally conscious and efficient future in aquatic food production. It has the potential to provide a more sustainable and ethical solution to meet the global demand for seafood while preserving marine resources and biodiversity.

Cell-Based Fish: A Novel Approach to Seafood Production and an Opportunity for Cellular Agriculture

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Cellular agriculture is defined as the production of agricultural products from cell cultures rather than from whole plants or animals. With growing interest in cellular agriculture as a means to address public health, environmental, and animal welfare challenges of animal agriculture, the concept of producing seafood from fish cell- and tissue-cultures is emerging as an approach to address similar challenges with industrial aquaculture systems and marine capture. Cell-based seafood—as opposed to animal-based seafood—can combine developments in biomedical engineering with modern aquaculture techniques. Biomedical engineering developments, such as closed-system bioreactor production of land animal cells, create a basis for the large-scale production of marine animal cells. Aquaculture techniques such as genetic modification and closed system aquaculture have achieved significant gains in production that can pave the way for innovations in cell-based seafood production. Here, we present the current state of innovation relevant to the development of cell-based seafood across multiple species, as well as specific opportunities and challenges that exist for advancing this science. The authors find that the physiological properties of fish cell- and tissue- culture may be uniquely suited to cultivation *in vitro*. These physiological properties, including tolerance to hypoxia, high buffering capacity, and low-temperature growth conditions, make marine cell culture an attractive opportunity for scaled production of cell-based seafood; perhaps even more so than mammalian and avian cell cultures for cell-based meats. This opportunity, coupled with the unique capabilities of crustacean tissue-friendly scaffolding such as chitosan, a common seafood waste product and mushroom derivative, presents promise for cell-based seafood production via bioreactor cultivation. To become fully realized, cell-based seafood research will require more understanding of fish muscle cell and tissue cultivation; more investigation into serum-free media formulations optimized for fish cell culture; and bioreactor designs tuned to the needs of fish cells for large-scale production.

Introduction

It is estimated that industrialized fisheries and fishing due to marine capture have lowered ocean biomass content by up to 80% (Myers and Worm, 2003). This effect, coupled with the effects of global warming on oceans, threatens to decimate wild fish populations (Funk and Brown, 2009). While some herald the rise of aquaculture as an ecological and economic boon (Tidwell and Allan, 2001), others feel that its benefits have been overstated and that it does not fundamentally solve current strains on wild fish populations (Naylor et al., 2000; Merino et al., 2012). At first glance, aquaculture seems capable of relieving the pressures of wild capture; however, this may not be the case. Carnivorous farmed fish are often fed wild fish, modifying natural habitats through harvest and cultivation (Naylor et al., 2000). While aquaculture was found to be a suitable means of feeding the earth's growing population, this would necessitate a reduction of wild-caught fish used for aquaculture feed, as many aquacultured fish eat fishmeal derived from wild-caught fish (Merino et al., 2012). Thus, even as aquaculture increases, so does wild-capture.

The challenges of feeding the world's growing population in a healthy way and in the context of sustainability have been presented (Lindgren et al., 2018). In this thesis, “Eating for the post-Anthropocene: Alternative proteins, Silicon Valley and the (bio)politics of food security,” novel animal proteins as a new locus for biopolitical disruption are presented as agents that affect culture and geographies (Sexton, 2018).

With oceans at risk, cell-based seafood provides a unique opportunity to transform the sustainability landscape. The concept of producing meat from cell cultures rather than from whole animals as a means to provide nutritional muscle tissue is no longer novel (Datar and Betti, 2010). The conversation around this concept has centered on the growth of mammalian or avian cells and tissues to replace meat, but cellular agriculture could easily be extended to fish, mollusk, and crustacean cells and tissues to replace seafoods. Such an extension could yield unique benefits that have not yet been explored. There are many considerations for cell-based seafood, with significant differences from those of cell-based land animals. These considerations include the scientific considerations of working with these unique cell lines, and human factors, including cultural, gustatory, and regulatory considerations, all fall within the broader framework of the necessity of this pursuit from a sustainability standpoint (Figure 1). While cell-based seafood shares some common ground with its land-based analogs in science and human considerations, sustainability is an even more substantial driver, as cell-based seafood could lead to greater preservation of marine environments.

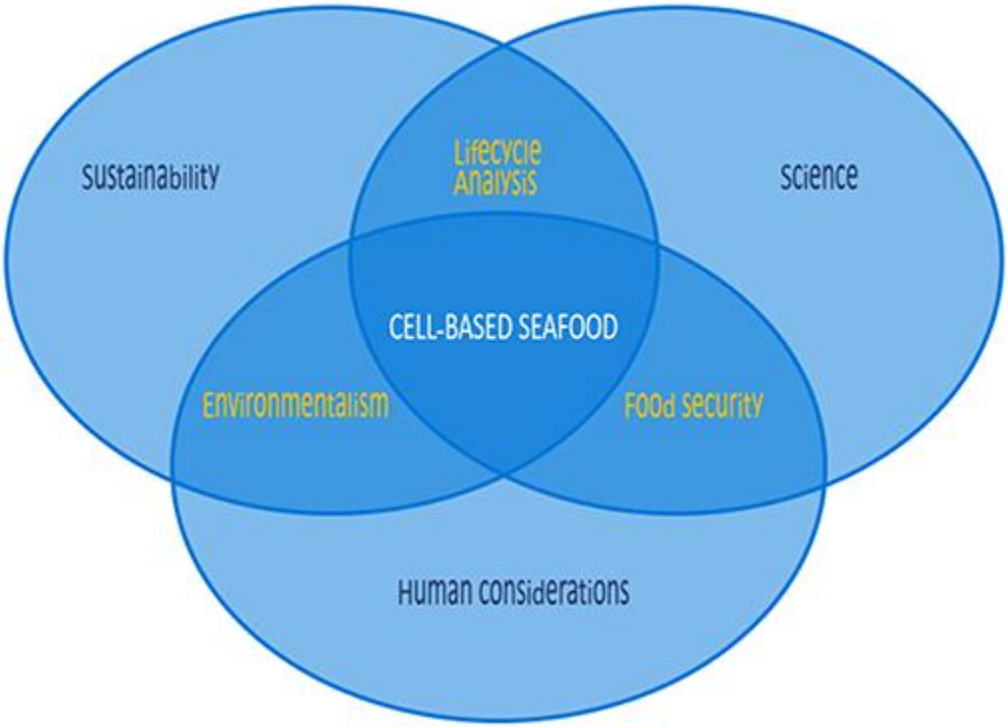

Figure 1. Considerations for cell-based seafood are numerous, differing in many respects from those of cell-based land animals. While cell-based seafood shares some common ground with land-based analogs in science and human considerations, sustainability is a more substantial driver, as cell-based seafood could lead to greater preservation of marine environments.

Preliminary life-cycle analyses indicate that cultured meat production could reduce greenhouse gas emissions by as much as 95% (Tuomisto and Teixeira de Mattos, 2011). However, the cost of these products remains to be seen as these products have not yet reached the market. There is much thought that initial product offerings will be more expensive than conventional meat. It is possible that the costs of cultured meat and fish production might lead them to become the food

of the elite, allowing these individuals the opportunity to enjoy the nutritional and food safety benefits, and in some cases, bypass the guilt of animal consumption (Stephens et al., 2018).

Certain considerations, such as safety regulation and cultural acceptance, are much harder to quantify, and there is reason to believe that the passage of time and increasing familiarity with cultured foods may support broader acceptance. Cultured meat adoption may prove easier and quicker in countries such as China and India, rather than in the United States (Bryant et al., 2019). There are other reports that indicate cultured fish may have a mixed reception initially, given most individuals prefer a beef burger or a plant-based burger to a cultured meat burger (Slade, 2018). Such preferences may shift over time, but they do pose short-term challenges for the field.

In addition, safety regulations are not fully established for cellular agriculture products in the United States, the United Kingdom, or the European Union, though a number of regulation-relevant topics were recently summarized (Stephens et al., 2018). Such regulations have not yet reached cellular aquaculture.

Trends in Seafood Production

While aquaculture is a very old technology—pictorial engravings suggest that Egyptians used aquaculture for fish as early as 2,500 BC (Anon—FAO, 1987)- it was only in the 1970s that aquaculture production became relevant beyond the subsistence and small-scale (Asche et al., 2008). Similar to the evolution of agricultural land animal farming, aquaculture has moved from extensive systems that depend on natural environments with minimal human intervention to semi-intensive and intensive systems, where producers actively control growing conditions through feeding, breeding, disease control, and waste removal, resulting in much higher production rates (Asche et al., 2008). With the intensification of aquaculture comes several challenges similar to the intensification of land animal husbandry, including the greater production of nitrogen and phosphorus waste materials from uneaten feed and metabolic waste products (Yeo et al., 2004). This creates a higher risk of negative environmental impacts (Piedrahita, 2003), such as the damage of waterways and promotion of algal blooms (Paerl et al., 2014). Intensification also increases the risk of pathogen spread from farmed fish to native species (Naylor et al., 2000). Furthermore, an absence of research on the welfare of most aquaculture species has made it difficult to develop welfare standards for farmed aquatic animals, and thus the ethical implications and appropriate welfare regulations of aquaculture remain an open question (Browman et al., 2018).

Aquaculture has recently surpassed marine capture as the main source of seafood for human consumption (Figure 2) (Food FAO, 2018). Global landings for marine capture have remained constant at around 90 million tons per year since 1994, while global aquaculture of fish and shellfish has nearly doubled over the same time period (Food FAO, 2018), thus, aquaculture has not relieved the demand for wild-caught seafood (Naylor et al., 2000). While aquaculture is often seen as mitigating overfishing, many farmed fish depend on feed originating from marine capture. Because several marine captured fish are used to feed a single carnivorous farmed fish, aquaculture may be decreasing the global fish supply, rather than increasing it (Naylor and Burke, 2005). While fully-controlled aquaculture environments are more efficient production

systems than the use of natural conditions (Tacon and Metian, 2008), to date, there is no scientific literature about fully-controlled environments for producing seafood from cell cultures rather than from marine animal husbandry.

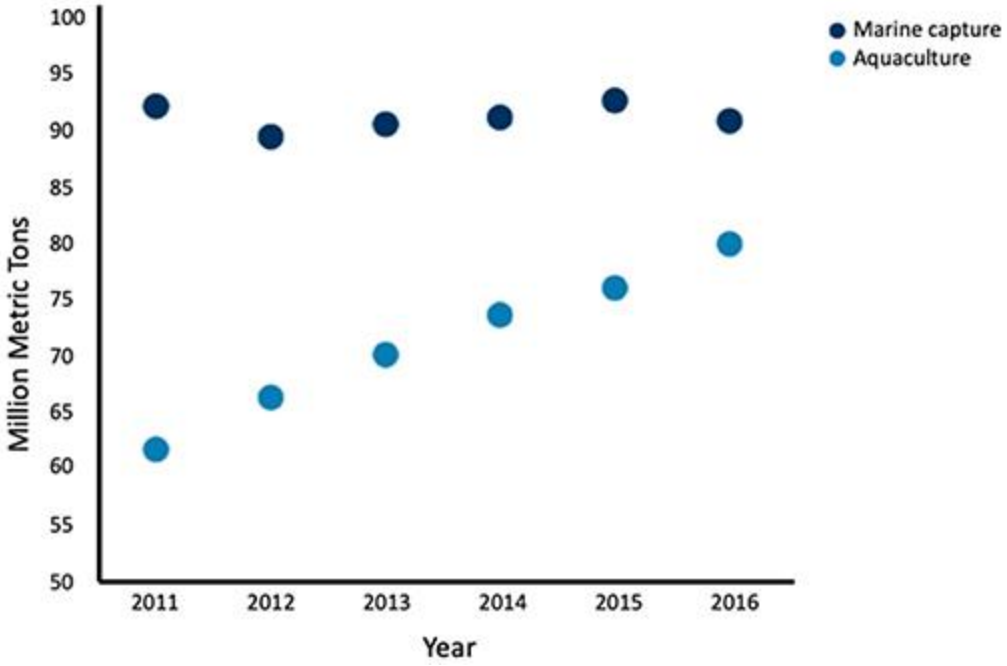

Figure 2. Global seafood production by marine capture and aquaculture by year from 2011 to 2016. Aquaculture production has increased while marine capture has remained fairly constant. Fish, crustaceans, mollusks, and other aquatic animals are included, and aquatic mammals, crocodiles, caimans, seaweeds, and other aquatic plants are excluded. Data adapted from the FAO (2018).

Engineering Biology for Seafood Production

Genetic modification has resulted in spectacular production increases, feed conversion rates, and reduced development times for fish in closed systems (Muir, 2004). Genetically-modified rohu develop over four times faster than their traditionally bred counterparts (Venugopal et al., 2004), while genetically-modified mud loaches are able to grow 35 times faster and substantially larger (Nam et al., 2001). Like the mud loach, genetically-modified tilapia containing a salmon growth hormone spliced to an ocean pout antifreeze promoter gene can grow over 320% larger than similarly reared non-transgenic fish (Rahman et al., 1998). The OPAFPcsGH gene, which encodes for regulatory elements from ocean pout followed by growth hormone, forms the "all fish" chimera gene sequence used in Aquabounty salmon (Hew and Fletcher, 1996). Aquabounty salmon, approved for manufacture and sale in Canada, develop twice as fast as their wild type counterparts with an impressive feed conversion rate 10% higher than farmed salmon (Bisson, 2015). These fish are formidable food sources and an ecosystem threat—and thus are produced only in closed, in-land systems as triploid, and therefore infertile females (Reichhardt, 2000). Despite these safety measures, significant regulatory hurdles, coupled with legislative pressure, have prevented the fish from entering the US market (Waltz, 2017). These fish are a good example of the power of engineering biology to improve closed-system seafood production.

These genome-level advances for closed aquaculture systems may lay important groundwork for cell-based fish production research and development.

Cell-Based Seafood Production

The Progression of Cellular Agriculture

In the past 5 years, research focused on cell-based meats has accelerated from the tasting of the cell-cultured hamburger in 2013 (Zaraska, 2013) and early publications, including life-cycle assessments and basic research (Tuomisto and Teixeira de Mattos, 2011; Post, 2012, 2014), to a growing start-up community, updated life cycle assessments, and publications focused on refining the technologies required to accelerate cell-based meat production (Tuomisto et al., 2014; Krieger et al., 2018; Rubio et al., 2019). To date, research decisions in cell-based meat production, such as selection of cell species and cell type, have been largely driven by market size and environmental impact (Rodriguez-Fernandez, 2019), rather than the suitability of cell species and types suitable for large-scale bioreactor cultivation. This is the result of a general lack of basic research on the cell cultures of commonly consumed animals.

Cellular agriculture has recently become a topic of interest for regulators in the United States. Given the regulatory jurisdiction of the Food and Drug Administration (FDA) over products of biotechnology and the United States Department of Agriculture over livestock, there is interest in the regulations necessary for potential jurisdiction over cell-based meat products (Stephens et al., 2018). Despite relative clarity over seafood, which is regulated entirely by the FDA (except for animals in the order Siluriformes) (Kobbeman, 2004), cell-based seafood production, and its potential to address a growing demand for seafood while avoiding the challenges of industrial aquaculture, has remained relatively uninvestigated from a regulatory standpoint.

Basic Methodology for Cell-Based Seafood Production

As with cell-based meat production, cell-based seafood relies on advanced technological developments in the optimization of cell lines, media formulation, and bioreactor design (Figure 3) (Datar and Betti, 2010). Like any closed cell or tissue-culture system, a cell-based seafood production system would consist of the following integrated elements: appropriate cell type(s) from the tissue of interest; a growth medium to provide nutrients to proliferating and differentiating cells, and a bioreactor to provide the closed environment to support the growth. For three-dimensional tissues, a biocompatible scaffold would be needed to provide structure for cell growth and maturation. A muscle protein production system (mpps) for goldfish was developed in 2002 with NASA funding (Benjaminson et al., 2002), however, there are no other examples of cell-based seafood production published during this time period.

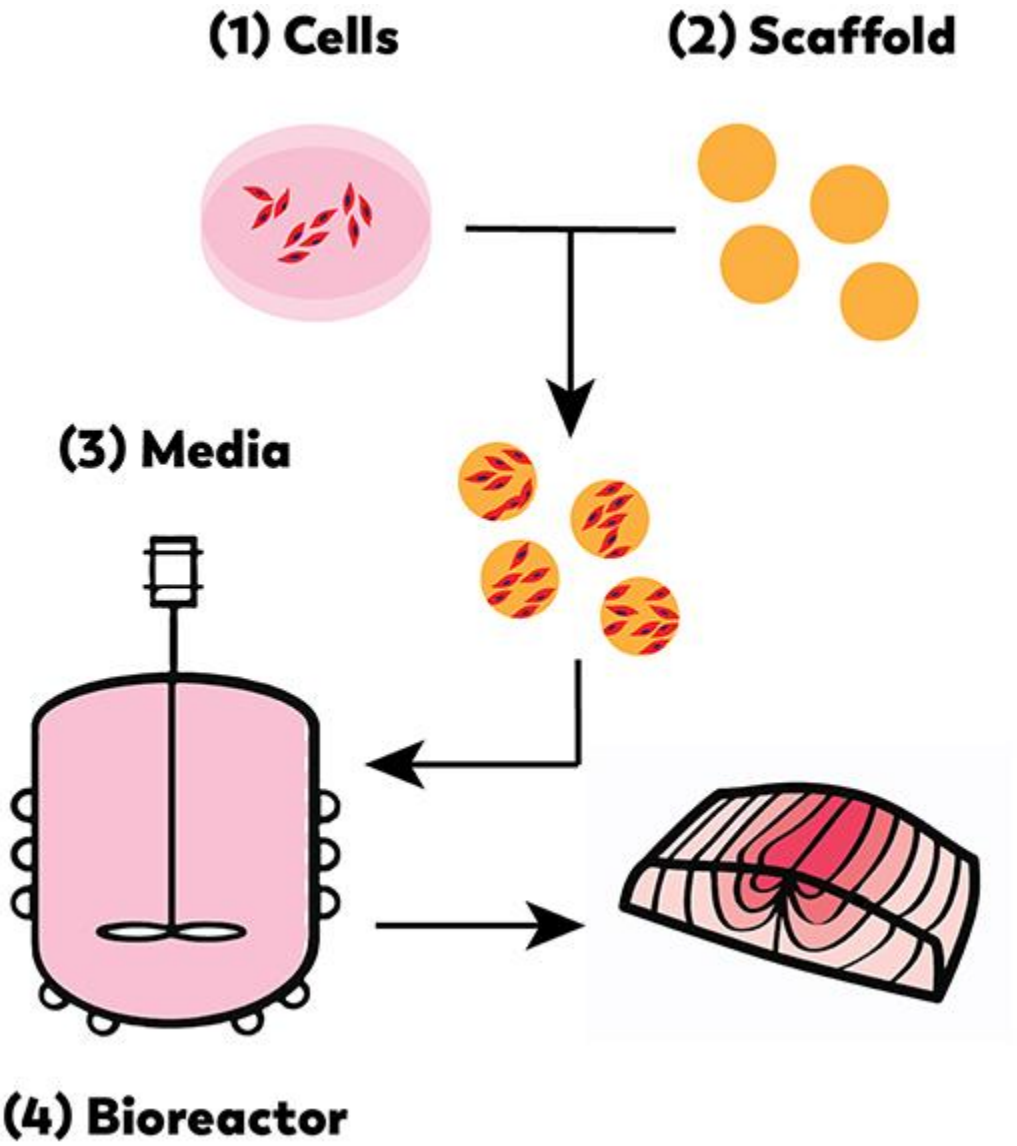

Figure 3. Cell-based seafood production will require the appropriate: (1) cell line(s), (2) compatible scaffold, (3) medium, and (4) bioreactor (Edelman et al., 2005).

Bioreactors are complex closed-system environments for producing biomass that require the constant monitoring (feedback loops for process control), maintenance, and optimization of several parameters. Marine cell cultures may be more forgiving in terms of temperature, pH, and oxygen requirements compared to mammalian cell cultures, based on the unique characteristics of native fish muscle tissue. This difference has significant implications for energy use and, thus, cost considerations for mass production of cell-based seafood. These differences also present a new set of research opportunities for cellular agriculture and toward large-scale tissue engineering in general; most medically-motivated tissue engineering is focused on generating smaller-scale tissues, usually no larger than organs (Griffith and Naughton, 2002).

Characteristics of Native Fish Muscle Tissue Compared to Mammalian Cell Culture

Disciplines such as bioengineering, cell biology, and genetics have made significant gains in the understanding of cell and tissue culture over the past 20 years; however, these advances have been mostly focused on mammalian systems. Cells and tissues from fish and other marine species have not yet been widely investigated *in vitro*. In contrast, native fish muscle physiology has been relatively well-researched because of the implications for aquaculture. Some experimentation and assays have been carried out on harvested native muscle tissues from freshwater and marine animals (Castellini and Somero, 1981; Eberlee and Storey, 1984; Anchelin et al., 2011), and these studies provide interesting insights into the potential capabilities of fish muscle in culture.

Native fish muscle consists of three muscle types—each of which differs between species depending on fish type, location, and feed (Kiessling et al., 2006). In teleost and elasmobranch fish, there are three muscle types: red, white, and pink. Red muscle is highly vascularized and is comprised of slow-twitch fibers with a high density of mitochondria and a rich supply of capillaries (Johnston, 2001). This muscle is for slow, sustainable swimming speeds and relies on aerobic metabolic pathways. White muscles are fast-twitch, tightly packed with myofibrils, and primarily utilize anaerobic metabolic pathways (Kiessling et al., 2006). White muscle is used for burst swimming and fast starts. Pink muscle shares some of the characteristics of both white and red muscle (Johnston, 2001).

The variety of fish muscle cell types, which vary in physiology and metabolic pathways, may offer a range of options when designing closed cultivation systems for cell-based seafood production. Native fish muscle also has characteristics related to metabolism, as described below, that, if exploited in *ex vivo* tissue culture, would make this tissue source uniquely suited for cell-based production.

Oxygen Requirements

Mammalian Cell Culture

Low oxygen concentrations are detrimental to mammalian cell growth and recombinant protein production (Wang et al., 1994), and thus dissolved oxygen concentration and air saturation are important variables that must be monitored and maintained in bioreactor systems. Air saturation levels of 40–60% are often required for mammalian cell culture (Furukawa and Ohsuye, 1998; Link et al., 2004; Zhu et al., 2005). Air saturation and oxygen levels become even more critical in three-dimensional, thick vascularized tissues where oxygen diffusion limits the development of tissues past about 1.8 mm (Griffith et al., 2005).

Fish Cell Culture

Fish are well-adapted to tolerate low oxygen environments as aquatic animals, as they are frequently subjected to low oxygen levels that result in hypoxic conditions (Vaquer-Sunyer and Duarte, 2008). Interestingly, many coral reef fish are tolerant of hypoxic conditions as low as 2.8–0.5% air saturation for the goby *Gobiodon histrio* and 1.6–0.7% air saturation for the blenny *Atrosalarias fuscus* before showing signs of distress (Nilsson and Ostlund-Nilsson, 2004). Using a microarray- and bioinformatics-based approach, over two hundred hypoxia-responsive genes were identified in the Japanese medaka brain, gill, and liver, ranging from cell metabolism to RNA processing to protein degradation through the ubiquitin system (Zhang et al.,

2009). For instance, Hif-1-alpha is a transcription factor that is present under hypoxic conditions–when molecular oxygen is present, the protein becomes hydroxylated and subsequently degraded by the ubiquitin system (Ivan et al., 2001). This highly tuned system, discovered in mammals, plays a notable role in the hypoxia response of fish. Analysis of icefish mRNA revealed that the functional domains of Hif-1-alpha are highly conserved, indicating continued selective pressure exerted by hypoxia (Rix et al., 2017). These and other hypoxia response gene relationships have been characterized in detail in the hypoxic fish database, HRGFish, which provides a variety of prediction tools and validation methods to further evaluate the conservation of hypoxia genes in diverse fish species (Rashid et al., 2017).

In the absence of research on the oxygen requirements of fish cell culture in bioreactor systems, the authors speculated that the genetic adaptations of fish to the levels of hypoxia suggest that fish tissues may be uniquely suited to the oxygen-limited environments found in tissue culture and provided via bioreactor conditions. Further, the presence of hypoxia response genes that are conserved in mammals suggests potential targets to increase the ability of mammalian cells to tolerate low oxygen environments.

pH Considerations

Mammalian Cell Culture

Extracellular pH correlates with intracellular pH in mammalian cell culture (Lagadic-Gossmann et al., 2004), while lactic acid, which is formed by cells in anaerobic conditions (Brooks, 2009), accumulates over extended periods of culture in bioreactor systems (Zhao and Ma, 2005). This accumulation contributes to the acidification of the bioreactor environment. The well-documented relationship between intracellular acidification and cell death (Lagadic-Gossmann et al., 2004), and because reduction of extracellular pH from 6.8 to 6.3 can render cells quiescent (Taylor and Hodson, 1984), pH control of bioreactor systems requires fine-tuning to optimize cell growth. Shifting the pH can also be used as an optimization tool, as changes in pH can affect protein production and glycosylation (Borys et al., 1993); however, altering pH to increase protein production and process performance usually reduces cell growth and metabolism (Trummer et al., 2006). For mammalian cell cultures, the pH of a bioreactor system must be actively maintained to counter the build-up of acidic metabolites like lactic acid, for either cell proliferation or protein production, depending on the goals of the culture system.

Fish Cell Culture

A key physiological characteristic of *in vivo* muscle across species is intracellular buffering capacity (Burton, 1978), the ability of a cell to maintain a neutral pH in the presence of metabolic end products such as lactic acid (Rogatzki et al., 2015). Intracellular buffering capacity is measured in Slykes, or micromoles of base required to change the pH of homogenized tissue by one unit per gram of wet weight (Slyke, 1922).

Land mammals such as pigs have buffering capacities of 49.7 Slykes, and cows 51.9 Slykes, while sea mammals such as the fur seal have buffering capacities of 79.2 Slykes, and spotted dolphins 84.1 Slykes (Castellini and Somero, 1981). White muscle in warm-bodied fishes has particularly high buffering capacities; 102 Slykes in the black skipjack, and 107 Slykes in the albacore, or double that of many land mammals (Castellini and Somero, 1981). Further, muscle

lactate dehydrogenase (LDH), the enzyme responsible for breaking down lactic acid in muscle tissue, exhibits up to 2-fold higher activity in warm-bodied fish such as the albacore when compared to land animals such as the cow (Castellini and Somero, 1981).

The higher intracellular buffering capacity and the higher muscle lactate dehydrogenase activity unique to warm-bodied white fish muscle *in vivo* suggest that these muscle tissues may be more resilient than mammalian cells in terms of *in vitro* tissue culture, with an ability to grow well over a wider pH range. More research is required to determine if the buffering capacity of native muscle tissue correlates with the buffering capacity of these tissues when cultured *in vitro*.

Temperature Requirements

Mammalian Cell Culture

Mammalian cells in culture are most often maintained at 37°C, human body temperature, a key factor in mammalian bioreactor process design. However, the viability of mutant Chinese hamster ovary cells was improved by alternating culture temperatures between 39 and 34°C (Jenkins and Hovey, 1993), while cultures grown at 30°C can live longer and produce more protein (Moore et al., 1997) than those cultivated at 37°C.

Fish Cell Culture

Fish vary widely in their adaptation strategies to frigid arctic and Antarctic environments, speciating at a higher rate near the Earth's poles than near the equator (Rabosky et al., 2018). Some of these adaptations have resulted in the development of antifreeze defense systems that are optimized for temperatures of 0° to 10°C (Abele and Puntarulo, 2004) and an increased production of antioxidants, such as marine-derived tocopherol, which is found in greater quantities in salmon from cold waters than fish from equatorial waters (Yamamoto et al., 2001). Other adaptations include unique oxygen-carrying and metabolic capabilities (Verde et al., 2008). In some cases, these adaptations manifest as even the lack of hemoglobin, as is the case in the demersal icefish, *Chaenocephalus aceratus*; these fish compensate for their lack of hemoglobin with increased numbers of mitochondria (Johnston, 1987). Adaptations in arctic species exhibit muscle design and mitochondrial density similar to high-performance adaptations in more equatorial species (Pörtner, 2002). Fish cell culture conditions typically mirror those of their typical habitats, with culture temperatures varying from 15 to 30°C. Some fish cell lines can vary their rate of metabolism within a 5°C temperature range, with the cells metabolizing more quickly at higher temperatures (Courtenay and Williams, 2004). Since fish cells can be cultured at cooler temperatures, this reduces the energy required to maintain a constant temperature of a culture system and the costs associated with producing cell-based seafood at scale. Thus, cell-based seafood may offer cost benefits at scale compared to cell-based meats.

Fish Cell Lines

Until recently, fish cell lines have found limited applications, mainly to test viral load, to examine water toxicology, and to generate vaccines for farmed fish (Wolf and Mann, 1980; Lai et al., 2008). Few of these cell lines have been utilized to produce edible fish, except for a study focused on producing fish-based proteins for astronauts (Benjaminson et al., 2002). The

American Type Culture Collection (ATCC) contains over 4,000 cell lines; however, it does not currently contain any fish muscle cell lines (Hay et al., 1992).

Few cell line databases include fish cell lines, while two (FICELdb and Cellosaurus) do so. FICELdb is a fish cell database that refers to many legacy lines dating from 1962 to 1999 (Braga et al., 2006). Cellosaurus is a cell line compendium created in 2012 and published in 2018, housed within ExPASy, a database maintained by the Swiss Institute for Bioinformatics (Bairoch, 2018). It consists of cell line repositories from around the world, categorizing over 100,000 known cell lines (Artimo et al., 2012). Interestingly, only 558 are immortalized fish cell lines, only nine are lines of fish cells isolated from fish muscle, none of which are myoblastic, all of which have spontaneously immortalized (Bairoch, 2018). While it is likely that not all immortalized fish cell lines are represented in these databases, the nine fish cell lines identified in Cellosaurus are displayed in Table 1.

Cell Line	Publication	Morphology	Water
White sturgeon	Wang et al., 2003	Epithelial	Salt
Bluefin Trevally	Zhao and Lu, 2006	Epithelial	Salt
Barramundi	Lai et al., 2008	Fibroblastic	Euryhaline
Zebrafish	Kumar et al., 2016	Fibroblastic	Fresh
Goldfish	Rougée et al., 2007	Epithelial	Fresh
Snakehead Murrel	Zhao et al., 2003	Fibroblastic	Fresh
Indian Catla	Ahmed et al., 2009	Epithelial	Fresh
Indian Catla	Ahmed et al., 2009	Fibroblastic	Fresh
Helicopter catfish	unpublished	Unknown	Fresh

Table 1. Immortalized fish muscle-isolated cell lines available from Cellosaurus.

Two of the nine fish cell lines originated from saltwater species, both of which are epithelial in nature: a white sturgeon line spontaneously immortalized in 2003, and a bluefin trevally line from 2006. The cell line originating from barramundi, a euryhaline fish, is fibroblastic. The remaining six fish cell lines originate from freshwater species. Zebrafish *Brachydanio rerio* and Goldfish *Carassius auratus* are commonly-known model organisms and pets; the remaining cell lines originate from commonly cultivated species in Southeast Asia: the snakehead murrel, a common food fish in Thailand; the Sahul Indian catla, a farmed fish that is often grown with other carp species; and the helicopter catfish, a species farmed commercially in Malaysia. Of interest, a goldfish cell line, while fibroblastic in nature, was successfully utilized to grow edible fish filets (Benjaminson et al., 2002).

While these nine cell lines have been useful for culturing a number of fish pathogens, none have yet been used for bioengineering or tissue engineering applications, and none are myoblastic lines. Derivation and characterization of myoblastic cell lines from various fish would be an important resource for researchers advancing cell-based seafood production. The availability of

fish cell lines that are well-characterized could be invaluable for cell-based seafood research, similar to the availability of C2C12 cells and their impact on mammalian muscle tissue engineering (Burattini et al., 2004).

Cell Isolation Methods

Lack of fish cell lines means that most fish tissue culture work is conducted on primary cells isolated from fish. Fish cell isolation methods are similar to mammalian cell isolations (An Introduction to Mammalian Cell Culture, 2016). In general, the fish is initially sterilized in ethanol, anesthetized, and a tissue sample is removed with a biopsy. The tissue sample is then either explanted or enzymatically digested with collagenase or trypsin. Explanted tissues are allowed to attach to the culture plate, and cells migrate from the tissue to the culture surface. Culture plates can be coated with proteins to enhance cell adhesion (e.g., gelatin, collagen, laminin). Enzymatically-digested tissues are in prepared aqueous solutions, where digestion by trypsin or collagenase releases the cells into the liquid medium. The cells are then rinsed with buffer to remove contaminants and are often filtered to liberate the cells from the residual debris (Oestbye and Ytteborg, 2018).

While these techniques work well for isolating tissue from adult fish, recent research has begun investigating the isolation of cells from embryonic teleosts (Anchelin et al., 2011). Embryonic cells from many wild species and wild fish that are artificially fertilized at fish hatcheries are challenging to isolate. Additionally, many species of fish have high telomerase activity, so primary embryonic cells do not senesce as quickly as in mammalian systems (Anchelin et al., 2011). Importantly, while fish do not have embryonic stem cells *per se*, they do have embryonic progenitors that may be coaxed down different differentiation pathways (Ghosh et al., 1997; Ma et al., 2001; Ciarlo et al., 2017).

As more research is carried out on fish muscle cell types, it should be possible to isolate cell types and populations of interest. Magnetic beads coupled to muscle-progenitor-specific antibodies can facilitate rapid isolation of these cells with minimal damage (Plouffe et al., 2015). Additionally, fluorescence-activated cell sorting (FACS) is an effective technique for cell isolation. The main challenge is identifying cell-surface proteins that distinguish these progenitor and muscle cells, particularly because the conservation in sequence with mammalian extracellular proteins is very low (Liongue and Ward, 2007), so most mammalian antibodies do not cross-react with fish-derived cells, thus limiting isolation and identification options.

Culture Conditions

Extracellular Environments and Scaffolds

The extracellular matrix (ECM) that fish cells need to survive and proliferate *in vitro* is a key area of research interest; most mammalian cell lines are plated on tissue-culture plates coated with vacuum gas plasma, which makes the polystyrene surface more hydrophilic. Atlantic salmon primary muscle cells have been successfully cultured and differentiated on laminin-coated tissue culture plates (Oestbye and Ytteborg, 2018). However, fish cells may require surfaces or scaffolds of different ECM proteins, such as elastins, collagens, fibronectin, and laminin, to adhere and grow efficiently, and may also require fish glycoaminoglycans (Frantz et al., 2010; Arima et al., 2013). Optimizing these ECM proteins would also likely be critical to

efficient fish culture *in vitro*, especially since fish protein glycosylation patterns are different from mammals.

Growth Media

Variables to consider for fish cell culture growth media include salt concentration, buffer, temperature, carbon source, and pH. Past growth media formulas suggest a trial and error approach that may not be optimal, including the use of mammalian or insect cell formulations such as Eagle's Medium, Modified Eagle's Medium (MEM), Medium 199 (M199), and Leibowitz's 15 (L-15) medium (Fernandez et al., 1993). These media are fully defined and easily replicated, including the content of inorganic salts, amino acids, and basic nutrients for culture growth. Additional salts are also added for some marine species, such as the grunt (Clem et al., 1961). Fetal bovine serum (FBS) and fetal calf serum (FCS) are common additives (Arora, 2013). The use of bicarbonate in media formulations aids cell growth and buffering capabilities as well (Fernandez et al., 1993). The ability to control the buffering capacity of the media is important; some fish cells require growth at 5% carbon dioxide, but others utilize anoxic or standard oxygen tension (Fernandez et al., 1993). These conditions vary between species (and even between tissues from the same fish) but can be controlled for cell-based seafood production. The addition of growth factors, such as fibroblast growth factor (FGF2) has proven to be helpful for the growth of some muscle cell cultures, including epithelial tuna cells in L-15 media with 10–20% FBS (Bain et al., 2013). Attempts to lower the FBS content resulted in reduced cell proliferation, while the addition of vitamin E and fatty acids improved proliferation (Scholefield and Schuller, 2014).

Removing FBS or FCS from the media for cell-based seafood production would be advantageous due to the high cost, their animal sources, and their potential for carrying mammalian viruses and prions (Van der Valk et al., 2010). Investigations into the molecular components of fish serum should be useful to identify and then generate *in vitro* the required components to keep fish cells active in culture. Importantly, fats are nutritionally relevant in seafood, especially omega-3 long-chain polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Wall et al., 2010). More research is necessary to understand how media composition affects the nutritional quality and taste of cell-based seafood. EPA and DHA are normally produced by marine algae, and studies have focused on large-scale production of omega-3 fatty acids from algal biomass; these studies indicate that optimization of algal growth and EPA and DHA production has strong potential for optimizing the industrial production of PUFAs (Chauton et al., 2015; Hamilton et al., 2015) and their inclusion into the media and cells of *in vitro* seafood cultures. The potential for non-animal sourcing of these PUFAs is environmentally attractive, avoiding ethical considerations, reducing potential contamination of cell-based seafood with animal pathogens, and also significantly reducing costs.

Crustacean Cell Culture

Cell Lines

Crustacean cell culture research has been pursued for use in aquaculture and pharmaceutical industries for studying diseases affecting farmed seafood and to identify biologics with human clinical relevance (Zhao et al., 2003). Attempts have been made to establish cell lines from crustacean tissues to improve isolation and maintenance methodology, including the use of short-

term cultures obtained from repeated primary cell isolations (Rinkevich, 2005). Short-term cultures are sufficient for bench-scale research but not sufficient for cell-based seafood applications with long-term goals of mass production and commercialization; thus, studies into the long-term culture of these cells are essential.

To advance crustacean tissue culture for food purposes, new strategies for developing immortalized or long-term cell lines of relevant lineages, such as muscle and fat, are needed. Previous crustacean tissue culture studies have instead largely focused on ovarian epithelial and fibroblast cells (Luedeman and Lightner, 1992; Fan and Wang, 2002; Maeda et al., 2003). Studies that examined muscle, eye stalk, and hepatopancreas cells in crustaceans found poor survival and slow growth compared to the ovary-derived epithelial and fibroblast cells (George and Dhar, 2010). The most frequently studied crustacean genus is *Penaeus* , which includes shrimp and prawn species. Studies with this species have derived primary cell cultures from the ovary, hepatopancreas, nerve, lymphoid, and hematopoietic tissue (Rinkevich, 2005). However, to date, no continuous crustacean cell lines have been established (Rinkevich, 2005).

Significant effort is needed to derive and characterize crustacean cells for use in cell-based seafood research and development. It is possible that the establishment of such cell lines could also be relevant to the aquaculture and pharmaceutical sectors.

Cell Isolation Methods

Cell isolation procedures for crustaceans and vertebrates share some similarities in terms of animal age and digestion procedures. For example, cells isolated from younger “pre-molting” prawns have a higher success rate for survival and growth (Tong and Miao, 1996). This is comparable to how more proliferative cells are correlated with younger mammal and avian donors (Doumit et al., 1990; Mesires and Doumit, 2002). Cell cultivation studies often attempt to isolate from both explanted and enzyme-digested tissues. For the explant approach, which is better suited for less dense tissues, the target tissue is harvested from the animal, rinsed in buffer solution, dissected into small sections, and allowed to attach to a tissue culture surface. The cells within the explant then proliferate, migrate, and adhere to the substrate. For the enzyme digestion method, digestive enzymes (e.g., trypsin, collagenase) are incubated with the dissected explants to degrade the tissue into single cells in suspension. The slurry is often filtered to remove undigested tissue, and the enzymes are inhibited after a certain amount of incubation time to prevent cell damage. For cell isolations from most shrimp tissues, the explant method tends to be more successful than the enzyme-digestion method in terms of cell proliferation, although enzyme digestion is preferred for hepatopancreas tissues (Ma et al., 2017).

Culture Conditions

Variables such as temperature, pH, and osmolality differ between vertebrate and invertebrate cell culture. The incubation temperature for invertebrates is lower than for vertebrates and typically falls within the range of 25–30°C (Hink, 1979). The pH of shrimp and prawn cell cultures is typically within pH 7.0-7.6 (Ma et al., 2017). Osmolality can range between 472 and 760 mmol/kg depending on the hemolymph osmolality of the origin animal (Chen et al., 1986; Rinkevich, 2005). Carbon dioxide exchange is generally not necessary for invertebrate tissue culture because the basal media tends to be buffered by phosphates rather than sodium bicarbonate. While some of the reported crustacean cell cultures (e.g., *Penaeus monodon* gonad,

heart, and epidermis cells) reach confluency and survive for extended periods of time with routine media changes, the cultures often degenerate after a few rounds of subculture (Chen et al., 1986; Owens and Smith, 1999). This loss of vitality in culture during passaging can be due to many factors, but may be in part due to the sensitivity of the cells to the enzymes employed during passaging.

Like fish cells, crustacean cells can be cultured at lower temperatures (Toullec, 1999), suggesting an energy savings opportunity for large-scale bioreactor cultures compared to mammalian cells. Similarly, crustacean cell culture does not require carbon dioxide exchange (Ma et al., 2017), obviating the need for a common mammalian cell culture bioreactor parameter control system.

Growth Media

The basal medium for invertebrate cell culture commonly consists of Grace's medium or L-15 medium, though formulations such as M199 have exhibited superior effects in some studies (Ma et al., 2017). The standard for mammalian cell culture is basal media (e.g., Dulbecco's Modified Eagle Medium, RPMI 1640, DMEM/F-12) supplemented with animal serum from domestic species like cows, horses or chickens. Fetal bovine serum is also important for shrimp cell culture at a concentration of 10–20% (Ma et al., 2017). However, this strategy has not been sufficient to maintain crustacean cells in culture long-term, so supplemented media with marine-relevant factors such as lipid solutions or hemolymph extracted from various sea creatures have been explored (Chen et al., 1986; Rinkevich, 2005). Common supplements include shrimp or crab muscle extract and hemolymph from *Penaeus* species (Ma et al., 2017). These additions likely provide trace and critical growth factors not supplied by mammalian serum. Some growth factors have been identified to improve shrimp lymphoid and ovarian cultures, including epidermal growth factor and transforming growth factor beta (Ma et al., 2017).

More study is needed in the development of a sustainable, animal-free medium for the growth of crustacean cells. The emphasis on animal-based media likely reflects the lack of understanding of the fundamental metabolic and growth requirements for crustacean cells and tissues.

Mollusk Considerations

Buffering Capacity

Buffering capacity varies widely in mollusks, with some species exhibiting particularly high buffering capacity (Eberlee and Storey, 1984). The channeled whelk, *Busycotypus canaliculatus*, has an aerobic buffering capacity in the hepatopancreas of 79.4 plus or minus 17.2 Slykes, and a remarkable buffering capacity of almost 120 Slykes following 24 h of anoxic stress (Hetrick et al., 1981). While heart muscle tissues appear to have a lower buffering capacity, anoxic stress increases the buffering capacity to over 60 Slykes (Eberlee and Storey, 1984), a value that is still higher than that of many land animals. The high buffering capacity of mollusks suggests an intrinsic ability of mollusk cell cultures to tolerate a wider pH range than mammalian cell cultures; assumptions that will require additional research.

Cell Culture

Mollusk culture has been even more elusive than fish or crustacean cell cultures. While primary cell cultures have been attained, continuous cultures of mollusk cell lines have yet to achieve

sustained proliferation *in vitro* (Chen and Wang, 1999). This includes attempts at long-term cultures of clam and oyster lines with 138 unique media formulations (Hetrick et al., 1981). Some mitotic activity was observed in scallop cell cultures over 4 months (Odintsova and Khomenko, 1991), and in the surf clam, *Spisula soldissima*, although the media in the latter study contained fetal calf serum and whole egg extract (Cecil, 1969). An exception to the challenge of proliferative cultures is from abalone species cultivated for pearl production and hemocyte (blood cell) lines used for toxicological studies. The mantle cells derived from tissue explants of *Haliotis varia* have been successfully cultured for 370 days in Medium 199 containing additional salts, lactalbumin, kanamycin, sodium bicarbonate, and fetal bovine serum (Suja and Dharmaraj, 2005). The mantle of *H. varia* was grown in serum-free media containing whole-body extract (Suja et al., 2007). Even fewer mollusk muscle cells have been isolated compared to fish. Clam primary cardiomyocytes were grown up to a month (Hanana et al., 2011), and primary cultures of clam heart tissue originating from *Meretrix luxoria* were successfully cultured for over 5 months (Chen and Wang, 1999).

Mollusk cell culture has been understudied, with demonstrated challenges in establishing long-term, proliferative, food-relevant cell lines. The success in the abalone industry suggests the opportunities are there with sufficient focus on the cell sources and cultivation conditions.

Scaffolds for Three-Dimensional Tissue Cultivation

Cultivating three-dimensional tissues relies on the presence of a scaffold, a biocompatible material capable of supporting cell growth and differentiation by providing a suitable morphology, chemical, and structural template. Numerous scaffolding materials that are employed in medical tissue engineering could be relevant to biofabricated food, such as cellulose, alginate, and chitosan. Chitosan is of particular interest because it is edible, inexpensive, accessible, and well-referenced in tissue engineering literature. Chitosan is a food-relevant material that is derived from chitin, a primary component of insect and crustacean exoskeletons and one of the most prevalent biopolymers on earth (along with cellulose). Within exoskeletons, chitin exists as nanofibril structures, providing mechanical strength to the cuticle (Ifuku, 2014). Chitosan can also be derived from non-animal sources like fungi, algae, and yeast (Croisier and Jérôme, 2013). Chitosan powder can be prepared from crustacean, mushroom, or microbial sources after the isolation of chitin and the chemical deacetylation to generate chitosan. This material is then dissolved into an aqueous solution, which can be cast into a variety of formats such as membranes, hydrogels, and sponges (Croisier and Jérôme, 2013). Fungal chitosan is a preferred source as it is: (1) non-allergenic, (2) customizable in terms of molecular weight, and (3) approved for human consumption as a food additive or nutrition supplement (Pochanavanich and Suntornsuk, 2002; Wu et al., 2004; Nitschke et al., 2011).

Scaffold Fabrication

Chitosan can be dissolved in an acidic solution, such as 1–2% acetic acid in distilled water. The viscosity of the solution increases rapidly with an increase in concentration (Jana et al., 2013). Many scaffolds that support various cell cultures use chitosan solutions as low as 0.5% (Katalinich, 2001). To create films to support cell culture, chitosan solutions are cast on glass substrates and allowed to dry, then rinsed with buffer to neutralize the acetyl groups, washed with water or PBS, and sterilized with 70% ethanol and UV light prior to cell seeding (Rubio et

al., 2019). Chitosan can also be manipulated to produce physically associated or cross-linked hydrogels (Drury and Mooney, 2003), porous sponges with tunable mechanical properties and pore size distributions (Jana et al., 2013; Rubio et al., 2019), and dry or wet spun fibers (Croisier and Jérôme, 2013).

Discussion

Given the progression of aquaculture toward more intensive, controlled, and efficient systems, cell-based seafood production offers a new option to potentially avert the challenges associated with industrial aquaculture and marine capture. The opportunities and challenges in the field of cell-based seafood production were reviewed, covering aspects of marine cell culture, native marine muscle tissue, and marine animal considerations. Because of the minimal literature on marine cell cultures, some insights and speculations on the characteristics of marine cell cultures are provided, based on the unique properties of native muscle tissue in fish.

Overall, fish muscle tissue may be well-suited for bioreactor cultivation relative to mammalian muscle tissues, given the ability of fish tissues to: (1) endure hypoxic conditions, reducing the need for active oxygenation in oxygen-limited bioreactor environments; (2) tolerate pH ranges, potentially establishing a wider range of pH in which cell growth is feasible; and (3) growth at lower temperatures, potentially reducing heat transfer needs of bioreactor cultivation at scale and reducing production costs.

A review of the literature on crustacean cell culture indicates that there is very little research that is directly relevant to cell-based food production. A review of the literature on mollusks is even more sparse. This dearth in direct insight into marine organisms related to cell isolations, cultivation conditions, and related needs provides a unique opportunity for scientific activity to help propel the field forward, not only for the field of cellular agriculture, but also for novel marine bioproducts and unique metabolic insights with potential translation to benefit human health and the environment.

Approaches to cell-based seafood production can range from large-scale cell cultivation, resulting in large masses of seafood-relevant cells and tissues for applications in processed seafoods like surimi, to three-dimensional tissue cultivation, resulting in structured products more akin to filets. Chitosan is a promising, marine-relevant biomaterial for three-dimensional tissue culture applications. It is customizable, edible, and widely available, making it a suitable material to explore for cell-based seafood production, as well as for other tissue engineering and cellular agriculture applications.

There could be several advantages to producing seafood from cell cultures rather than whole marine animals. The production of cell-based seafood has the potential to alter many fundamental parameters considered immutable in food cultivation, including the production of inedible excess tissues such as bones, skin, shells, and scales, which are often discarded and can negatively impact the environment. Cell-based seafood may also shorten cycle time; cell cultures may require weeks to months to generate functional foods; while by comparison, genetically-modified Aquabounty salmon require 18 months to grow to market size, roughly half the time of a normal salmon (Waltz, 2017).

Conclusions

Producing seafood from marine cell cultures is a novel seafood production method and an intriguing opportunity for cellular agriculture. A survey of relevant literature reveals that marine cell and tissue culture is an enormously neglected field of research. Few cell lines are available from marine species, and none are directly relevant to seafood production. Despite the properties of marine muscle tissue that suggest this as a promising opportunity for bioreactor cultivation, the behavior of marine cells in large-scale culture environments remains speculative. There are several research gaps that exist for marine cell culture, alongside several opportunities that make these research gaps worth addressing. With growing interest in cellular agriculture as a means to produce meat, milk, eggs, and other animal proteins from cell cultures, and with the rapid intensification of aquaculture systems, the time is right to investigate the production of seafood without marine animals.

Cell-cultivated aquatic food products: emerging production systems for seafood

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Abstract

The demand for fish protein continues to increase and currently accounts for 17% of total animal protein consumption by humans. About 90% of marine fish stocks are fished at or above maximum sustainable levels, with aquaculture propagating as one of the fastest-growing food sectors to address some of this demand. Cell-cultivated seafood production is an alternative approach to produce nutritionally complete seafood products to meet the growing demand. This cellular aquaculture approach offers a sustainable, climate-resilient, and ethical biotechnological approach as an alternative to conventional fishing and fish farming. Additional benefits include reduced antibiotic use and the absence of mercury. Cell-cultivated seafood also provides options for the fortification of fish meat with healthier compositions, such as omega-3 fatty acids and other beneficial nutrients through scaffold, media, or cell approaches. This review addresses the biomaterials, production processes, tissue engineering approaches,

processing, quality, safety, regulatory, and social aspects of cell-cultivated seafood, encompassing where we are today, as well as the road ahead. The goal is to provide a roadmap for the science and technology required to bring cellular aquaculture forward as a mainstream food source.

Introduction

The availability of safe, high-quality food for the burgeoning world population continues to be a major challenge in light of the deterioration of natural resources, coupled with climate change. To feed the estimated 10 billion people safely and sustainably by 2050, the world will need to produce significantly more food [1, 2]. It is anticipated that global demand for meat will increase by 70% from today, and planetary resources will be insufficient to meet the demand of the world population by 2050 [3]. Within this larger global challenge, aquatic sources provide nutritional protein-rich foods, including omega-3-enriched sources of fatty acids and bioavailable micronutrients [4]. Stagnant levels of fish harvested from open water fisheries and the growing challenges with the sustainability of aquaculture systems are concerns [4]. To adequately feed the growing global population by 2050, increases in seafood production of 100% are projected as a need [5]. Decade-wise comparisons of global per capita consumption, capture fisheries production, and aquaculture production from 2000 to 2020 based on FAO data are given in Fig. 1. Hence, there is an imperative to establish alternative sources of fish and shellfish to effectively meet the growing global protein demand in the foreseeable future (2020–2050) [2].

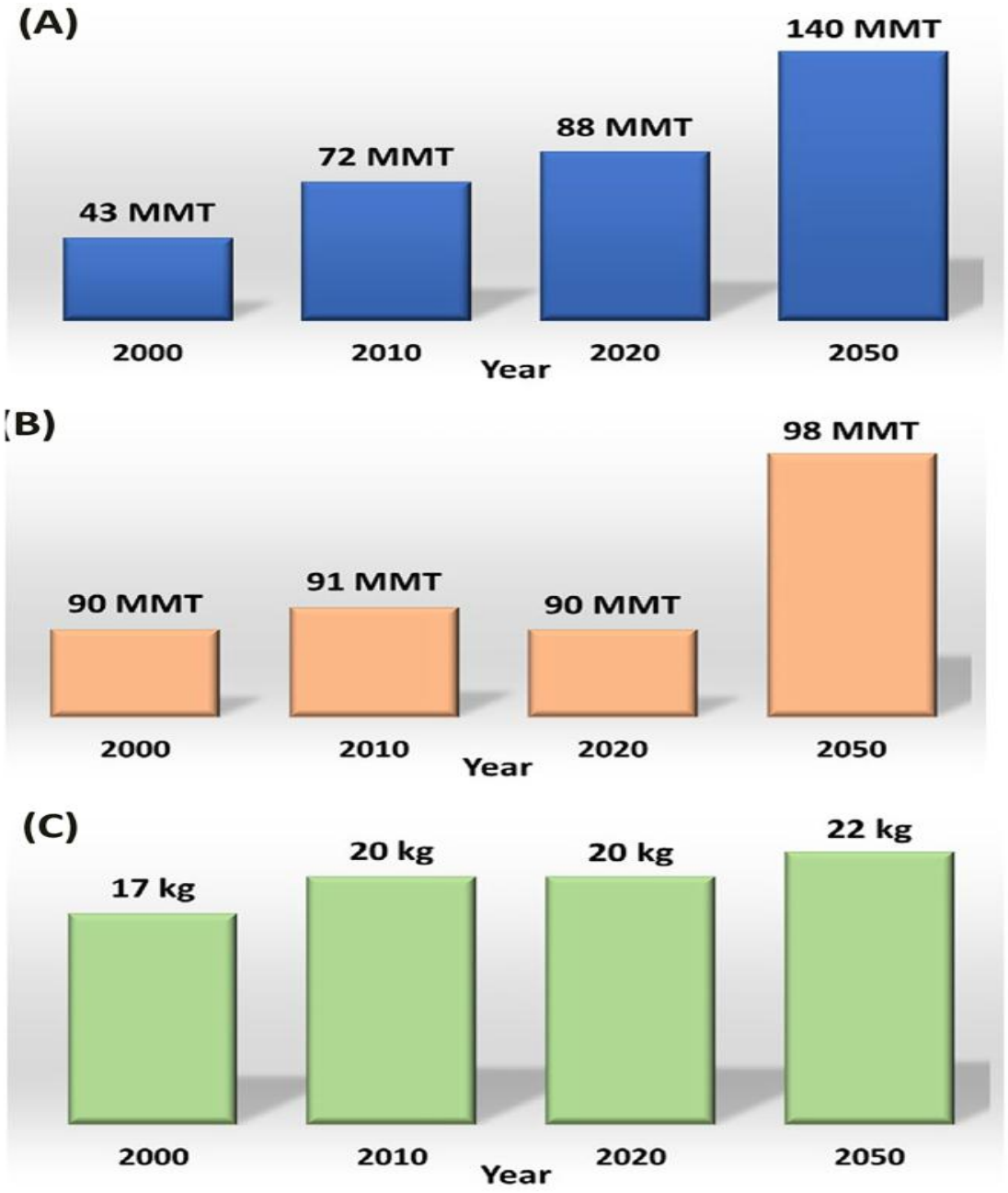
Presently, 89% of the aquatic animals produced—equal to 157.4 million tons—are used for human consumption, considering the per capita consumption of 20.2 kg of fish per year by 7.8 billion people. The rest is used mainly for non-food uses, including fish oil and fish meal production [4]. Future projections for capture fisheries and aquaculture production by 2050 are 98.3 and 140 million tons, respectively [4]. Thus, increases in future fish production will rely mostly on aquaculture production, which is challenging in the context of sustainable production. For fish production to be maintained at a sustainable level, critical efforts will be required to provide larger volumes of feed to support aquaculture, to maintain quality for aquatic environments, to reduce pressure on wild aquatic organisms used for food, and provide quality aquatic

foods to consumers [6,7,8]. These challenges prompt the development of alternative sources of aquatic food through cell-cultivated approaches.

Cell-cultivated seafood

Cell-cultivated seafood has gained attention as an alternative sustainable food production system, where animal cells are grown in vitro using cell culture techniques to form edible seafood products without the need for the animal [9, 10]. Cellular agriculture is one of the key transformative food production systems to help address the above challenges, which originated with the cultivation of goldfish in a study funded by NASA [9]. Cell-cultivated fish production requires the large-scale cultivation of cells to generate large masses of seafood-relevant cells and tissues. These cells and tissues can be used to form unstructured products such as surimi or fish fingers using well-established food processing techniques, or they can be further cultured on three-dimensional biomaterial scaffolds to generate structured products akin to fish fillets. The many advantages of producing seafood from cell cultures rather than using native fish include improved freshness, food quality, and avoiding non-edible components such as bones, skin, shells, and scales as waste that can negatively impact the environment. Cell-cultivated seafood may also shorten food production cycle time and provide continuous production; cell cultures may require weeks to generate functional foods and may do so in a continuous manner [10].

Fig. 1



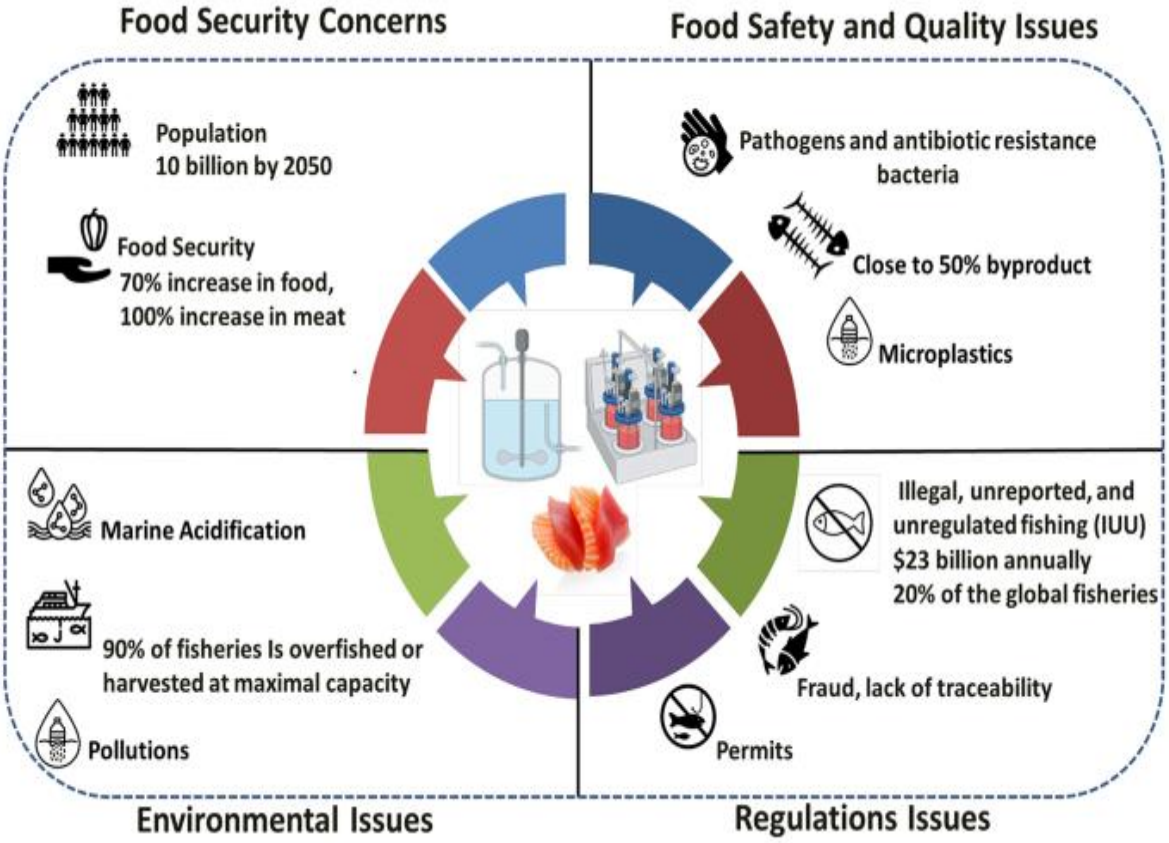
Trends and projection up to 2050 in total capture and aquaculture production and Consumption based on FAO Data [4]. (**A**) Global aquaculture production (million metric tons); (**B**) Global fisheries production (million metric tons); (**C**) Seafood consumption (kilograms)

Fisheries and aquaculture are relatively sustainable food production systems compared to terrestrial livestock, however, due to overfishing, pressure on wild stocks, emerging diseases, antibiotic-resistant bacteria, global warming, and marine acidification with adverse impact on organisms' physiology, loss of biodiversity and species migration, byproducts of production, microplastics, chemical contaminants in waters, and the lack of clean water (Fig. 2), the seafood industry requires alternative and innovative production systems to overcome these current challenges.

Research gaps and challenges

There are several gaps in research and development to be filled in order to progress cell-cultivated seafood.

Fig. 2



The main challenges in the seafood industry as driving forces for developing cell-cultivated seafood

Limited seafood cell lines: Producing seafood from fish cell cultures is an intriguing opportunity for cellular agriculture, yet few fish cell lines are currently available that have direct relevance to seafood production [10]. Cell-cultivated seafood processes rely on native seafood sources for harvesting muscle and fat cells, which are then immortalized. Both cell isolation and the immortalization processes remain challenging. For example, access to embryonic stages of many aquatic organisms as a source of stem cells is difficult. The number of cell sources has been expanding thanks to continuous research. Many of these sources, however, still need to be validated in a large-scale culture.

Limited knowledge of seafood cell differentiation: There remains limited knowledge in terms of in vitro fish, crustacean, and mollusk muscle cell or fat cell proliferation, differentiation, and maturation. Omics-based methods, including genomics, proteomics, and metabolomics, are helping to elucidate factors involved in the different stages of differentiation to accelerate cell-cultivated seafood production. Further, a number of studies with fish have provided insights into growth factor requirements and growth conditions [11]. Myogenic precursors from juvenile trout showed higher proliferation and differentiation rates than adult trout myogenic precursors [12], and insulin-like Growth Factor (IGF-1) and IGF-2 stimulated the proliferation in primary cell cultures of myoblasts from rainbow trout [13,14,15,16]. Gilthead sea bream (*Sparus aurata*) myocytes were cultured to evaluate the role of IGFs in muscle growth and differentiation via the regulation of myogenic regulatory factors (MRFs: MyoD, Myf5, myogenin, and MRF4) expression [17]. At the beginning of the cell culture and during the proliferation, the IGF-2 expression was highest. Additionally, further evaluations indicated that myod2 and myf5 expression (genes involved in early muscle cell proliferation) was increased by IGF-2, whereas IGF-1 increased mrf4 and myogenin expression (genes involved in differentiation) [17].

Lack of serum-free media: Serum-free media have been developed for mammalian cells, yet this remains a challenge for cell-cultivated seafood. Cell line development for seafood can require up to 20% serum, making cell-based seafood production unsustainable and expensive. Reducing serum can result in changes in morphology or slower to no cell growth [18]. Reduction of serum

in fish cell cultures has been achieved using IGF-2, algal extracts, and protein hydrolysates [18, 19], but elimination of serum without negative impact on growth remains a challenge [18]. More research is required to develop serum alternatives for cellular aquaculture, such as specific plants or bacterial/algal-based products.

Limited genetic tools: Exploring genetic modifications for seafood cells, to accelerate both understanding of cell proliferation and differentiation, as well as to develop cell lines, remains challenging due to the few genetic tools developed for seafood cells. Yet, optimization of immortalization and trans-differentiation processes through genetic modification, including CRISPR-Cas9 [20] editing of fibroblasts that convert them into skeletal muscle or adipose cells, will address some of the cell sourcing challenges for cell-cultivated seafood. Induced pluripotent stem cells are available for adult zebrafish [21], with limits to other publicly available seafood species. There remains limited knowledge of differentiation pathways in aquatic species other than zebrafish (*Danio rerio*) [21,22,23,24]. Genetic tools in other, traditionally consumed, species need to be pursued. Given that these technologies still require genetic modification, consumer acceptance and reactions to the consumption of genetically modified cells must be evaluated.

Scale Up Demonstration: Compared to mammalian cells, fish cells may be more suitable for bioreactor production due to their tolerance for hypoxic conditions, which reduces the need for active oxygenation; their increased tolerance for different pHs; and, in some cases, their growth at lower temperatures to reduce energy costs [10]. However, long doubling times are problematic, and scale-up data remains to be demonstrated.

Lack of available consumer-ready products: The inclusion of heme proteins in plant-based meat increased meat-like flavor and natural color [25]. Similar approaches are needed for aquatic cell-cultured foods to address consumer perceptions. The Peptide Atlas and Protein Map developed from Rohu (*Labeo rohita*) [26] is a useful source for identifying proteins involved in the quality and color of cell-cultivated seafood. Nutrition, flavor, texture, and quality of products and cultural relevance are important parameters that will need to be addressed for cell-cultivated seafood to achieve consumer acceptance as the

field progresses. Flavor in conventional seafood is mainly due to the fatty acids and some amino acids. Developing these flavors in the cultivated meat could be achieved by cell engineering to generate specific amino acids and fatty acids, manipulating cell culture media to contain more marine flavor-based compounds such as protein hydrolysates from marine plants, and adding flavor extracts to the final products.

Cell types for cell-cultivated seafood production

Developing cell-cultivated seafood starts by isolating embryonic stem cells, adult stem cells, or generating induced pluripotent cells from the species of interest [10, 14, 24, 27,28,29,30]. Despite efforts to establish cell lines from aquatic organisms (fish, mollusks, and crustaceans), the challenge remains to isolate and immortalize viable cells (Table 1).

Table 1: Examples of cell line development from aquatic organisms

Tissue selection is the first step for sampling, in the case of fish samples for myogenic cells, this often involves using white muscles with significantly less fat content compared to red muscles, however, the spatial arrangement differs among species (in most fish species, red fibers form a thin lateral superficial sheet just under the skin, whereas white fibers make up the underlying mass of the myotome). In order to isolate cells, adult tissue selection for mollusks plays a crucial role in establishing primary cell culture methods. Mollusks, such as oysters, have diverse tissue types that can dictate the culture conditions and cell dissociation methods [42]. Tissue from three main oyster species, Pacific (*C. gigas*), Eastern (*C. virginica*), and European Flat oyster (*Ostrea edulis*), have been studied for drug, toxicity, and disease research [30,31,32, 34, 42], including embryo, heart, mantle, digestive gland, gill, ventricle, and adductor tissues (Table 1). Among the oyster tissues studied, heart tissue was most frequently selected as it had better potential in establishing a permanent cell line than oyster embryos [31]. These previous studies indicate that the tissue of origin often dictates the success of oyster cell culture [42], along with culture conditions and decontamination treatments.

A significant challenge for seafood cell isolation is contamination from other species, particularly for marine filter-feeding bivalves such as oyster, mussel,

clam, and scallop [42]. Protozoans (*Thraustochytrium* sp.), amoeba, motile zoospores, sporangia, yeast, endospores, and microalgae are common contaminants in marine invertebrate cell culture [31, 42]. Finding optimal antibiotics and antifungal conditions during the initial cell isolation step is also challenging because high concentrations can damage or kill the desired cells, and low concentrations may not effectively eliminate the contaminating microbes [34, 42].

In order to develop cells suitable for bioprocesses for seafood, immortalized cells are required. Three methods of immortalization are generally pursued: spontaneous genetic processes, genetic modification approaches such as the expression of the catalytic subunit of telomerase (TERT), or genetic inactivation of p53/p14/Rb [43]. Spontaneous immortalization has benefits and limitations. For example, spontaneously immortalized cells are not considered genetically modified (GM), which allows companies access to European markets that restrict the use of GM foods [43]. However, this immortalization process is not controlled, thus additional genetic changes are feasible. In addition, every cell type has its own susceptibility towards spontaneous immortalization.

For example, fish cell lines have a higher susceptibility to spontaneous immortalization due to the high regenerative capacity of the adult stem cell population compared to mammals with more effective DNA repair mechanisms [44]. For cell-cultivated seafood production, spontaneous immortalized cell lines from Atlantic mackerel (*Scomber scombrus*) were developed [40], and a skeletal muscle cell line was confirmed through characterization of muscle stemness and differentiation via paired-box protein 7 (PAX7) and myosin heavy chain (MHC) immunostaining, respectively.

Importantly, an adipocyte-like phenotype was demonstrated for these cells through lipid accumulation from the environment, confirmed via Oil Red O (ORO) staining and quantification of neutral lipids, as an alternative path to adipogenesis utilizing adipose-derived cells. Limited antibody markers for fish-derived cells, including adipocytes and myocytes, continue to make cell identification a challenge for the field.

Cell growth conditions

Nutritional requirements in cell culture remain unclear for many cell lines from aquatic organisms. A simple basal medium with added artificial seawater (ASW) or sterile seawater (SSW) helped to provide osmolarity similar to marine habitats [42]. For example, for oyster cell culture media, osmolarity was adjusted to 650–720 mmol/kg[31]. The most common medium used for many aquatic organisms in cell culture is L-15, which contains salts, amino acids, galactose, vitamins, and minerals [30, 31, 34, 42]. However, the L-15 medium contains no proline or taurine, which are present at high levels in the body fluids or tissues of aquatic organisms [31]. Proline and taurine are likely essential components for cell proliferation in mammalian cells [45]. Therefore, adding proline or taurine to oyster cell culture media by using oyster body fluid or tissue extracts could be necessary for supporting cell proliferation. In addition to basal media, many media supplements and growth factors such as fetal bovine serum (FBS), adult organism soft body fluid, embryo or gonad extract, fibroblast growth factor (FGF), insulin, and epidermal growth factor (EGF) have been tested for cell proliferation, but with inconsistent outcomes [30,31,32, 34]. Different cell culture media, supplements, and incubation temperatures used for bivalve cell culture are presented in Table 2.

For oyster cell cultures, penicillin, streptomycin, and amphotericin B are the most commonly used antibiotics. The penicillin concentration for tissue decontamination ranges from 50 to 100 IU/mL, streptomycin ranges from 100 to 500 µg/mL, and amphotericin B from 0.25 to 2.5 µg/mL[31], [42]. These antibiotics were placed into Leibovitz's 15 (L-15) medium, phosphate-buffered saline solution (PBS), or artificial seawater (ASW) for washing the tissue samples. Other antibiotics such as ampicillin, gentamycin, and kanamycin have also been used [42]. It is important to note that decontamination might vary depending on the source of the tissues, as some tissues have a higher initial microbial load. For instance, digestive glands, gills, and the mantle are prone to more contamination than the heart or adductor muscle because these organs are primarily involved in filtration. In addition, some marine microbes and parasites carry a symbiotic relationship with the animal, leading to more contamination and making it difficult to find optimal decontamination conditions [42].

Table 2 Cell culture media, media supplements, and incubation temperatures used for bivalve primary cell cultures*

Aside from serum-free media needs, environmental factors such as oxygen, salt, pH, osmolarity, and temperature must be optimized. Fish cells are generally adapted to low oxygen environments with hypoxia-response genes [46]. Some fish cells only grow in 5% carbon dioxide, while others utilize anoxic or standard oxygen tension [47]. A comprehensive study on muscle lactate dehydrogenase (LDH) in warm-water fish and mammalian cells reported significant differences in metabolic activity dependent on pH [27]. Generally, seafood cells grow at lower temperatures than mammalian cells, making them good candidates for producing cell-cultivated seafood with lower energy inputs. There are different fully defined basal media available for seafood cell culture, including Eagle's Medium, Modified Eagle's Medium (MEM), Medium 199 (M199), and Leibowitz's 15 (L-15). While there have been significant advances in the development of serum-free culture media for mammalian cell lines, there has been limited progress for fish cells [27]. Serum-free media have been achieved for a few fish cell lines in the past; however, these formulations were not well-defined or were proprietary within companies, resulting in significant challenges in broadening their utility for cell-cultivated seafood.

Serum-free media containing lactalbumin hydrolysate, trypticase-soy broth, bacto-peptone, dextrose, yeast isolate, polyvinylpyrrolidone, and non-essential amino acids were studied with different fish cell lines (Table 3), and cell growth and morphology of the cells were similar to those that were grown in serum-containing media [48]. Bioprocessing was utilized to convert different feedstocks, including whole oysters (*C. virginia*), whole mussels (*M. edulis*), whole lugworms (*Arenicola marina*), black soldier flies (*Hermetia illucens*), and crickets (*Acheta domesticus*), to protein hydrolysates for growing fish cells [18].

These hydrolysates were cytotoxic for Zebrafish cells (ZEM2S CRL-2147™) at high concentrations (1 and 10 mg/ml) regardless of serum concentration, while, at lower concentrations (0.001-0.1 mg/ml), all of the hydrolysates supported cell growth. Black soldier fly hydrolysates could replace serum and provide a cost-effective source of peptides.

The use of modeling tools also has the potential to foster more rapid identification of key media and related conditions for seafood cell growth and differentiation. For example, through the use of Design of Experiments (DoE) and/or AI, the development of a serum-free medium can be pursued.

Table 3: Feedstocks and processing methods for fish cell growth

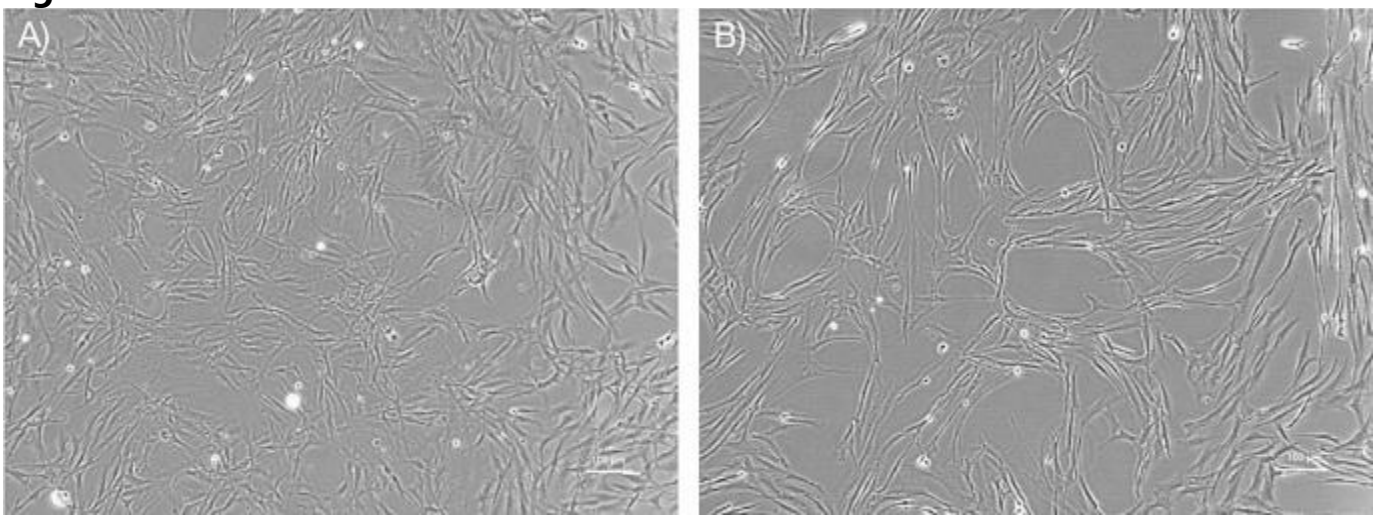
Protein hydrolysates from marine byproducts could also provide inexpensive and high-quality proteins and amino acids to develop serum-free media.

Differentiation – myogenesis and adipogenesis

There is limited knowledge on the in vitro differentiation and maturation of fish, crustacean and mollusk cells into fat or muscle tissues [40]. To screen for myogenesis in mackerel cells as an example, a variety of methods were utilized [e.g., serum starvation, reduced serum, reduced serum plus additives [50], reduced serum with insulin, 1-oleoyl lysophosphatidic acid (LPA) and transferrin, reduced serum medium with insulin-like growth factor 1, reduced serum medium plus additives with IGF-1; medium with extracellular signal-regulated kinase inhibitor (ERKi) [51]]. Myogenic potential was assessed via RT-qPCR using primers based on genome sequences from southern bluefin tuna (*Thunnus maccoyii*) (e.g., myogenic differentiation 1 (*MYOD1*), myogenin (*MYOG*), troponin T type 3a (*TNNT3A*)), along with immunohistochemistry.

Differentiation via paired-box protein 7 and myosin heavy chain immunostaining was observed in a continuous muscle cell line developed from Atlantic mackerel (*Scomber scombrus*) [40]. The cell line also exhibited an adipocyte-like phenotype, which was confirmed via Oil Red O staining and quantification of neutral lipids. MEF2A, Mrf-4, MyoD, and Myf-5 expression was reported in a muscle cell line developed from a freshwater fish (*Labeo rohita*) during differentiation of muscle cell culture [52]. However, more detailed studies need to be carried out to facilitate the selection of the right cell type for cultivated aquatic food development. Images of mackerel cells are provided in Fig. 3.

Fig. 3



Example images of mackerel cell lines. Phase contrast images: **A**) Mack1 Passage 147 and **B**) Mack2 Passage 124. Scale bars 100 μm. Photos provided by Michael Saad, based on research from https://doi.org/10.1038/s41598-023-31822-2 [40]

Scaffolds and tissue engineering

While suspension culture-based approaches may be sufficient for unstructured seafood products like surimi, tissue-like products that replicate some of the complexity of muscle tissue, including texture/mechanics and mouthfeel after cooking and oral mastication, will require more sophisticated methods to impart structure to the final product [53]. A variety of approaches are utilized that mainly rely on scaffolds to facilitate the transport of oxygen, nutrients, and waste products as tissues mature (Table 4). Approaches to scaffolding and tissue engineering for cell-cultivated meat have been reviewed elsewhere [54,55,56,57].

The differences in requirements for scaffolds for seafood vs. terrestrial meat can be divided into two broad categories: those related to the cell requirements and those related to the effects of the scaffold on the organoleptic properties of the final product. Because scaffolds play a crucial role in delivering cues to the cells as they proliferate, differentiate, and mature, scaffolds that are appropriate for use with cells from one taxonomic group may not be optimal for another. Therefore, optimization of scaffold stiffness [58], topography [59,60,61], or surface functionalization [62] may require

significant differences between terrestrial animals, fish, and aquatic invertebrates.

Scaffolds can also impact the acceptability of the final product due to texture, taste, and flavor. For example, the melting temperature of fish collagen differs from that of collagen from terrestrial animals, with important impacts on cooking fish muscle [63], thus, the thermal properties of scaffolds for cell-cultivated seafood will need to be carefully considered. In addition, the 3D geometry of muscles from terrestrial animals, fish [63], crustaceans [64, 65], and mollusks [66, 67] is different and needs design considerations with scaffolds for whole-cut cell-cultivated products.

One of the earliest investigations into cell-cultivated meat or seafood was a NASA-funded study that demonstrated the in vitro expansion of goldfish muscle explants co-cultured with brown bullhead fibroblasts [9]. While research into cell-cultivated seafood over the subsequent two decades has lagged behind that of cell-cultivated terrestrial meat, several recent studies have demonstrated progress.

Table 4 Scaffold considerations for cell-cultivated seafood

While this is an early example of a scaffold-free cell-cultivated seafood prototype, there is precedent for the use of scaffold-free techniques in both academic [68, 69] and commercial [70] efforts at producing cell-cultivated terrestrial meat, but less so for seafood-related goals. Recent advances with scaffold-free alternatives were reported for livestock-derived adipocyte cell cultures in 2D, which could also be applied to seafood cell cultures; the 2D systems were consolidated into 3D tissues via post-cell growth aggregation using food-grade cross-linking enzymes like transglutaminase or a gelling agent (e.g., alginate) [71].

The use of 3D bioprinting to produce cell-cultivated meat products has been a focus [72] due to the level of control over structure, and this strategy was also applied for the formation of cell-cultivated large yellow croaker (*Larimichthys crocea*) prototypes by printing with a bioink consisting of gelatin, alginate, and primary croaker satellite cells into a tissue-like structure [73].

Microcarriers (MCs) as scaffolds in suspension are also utilized towards cell production goals and scalability in cell-cultivated seafood production, providing large surface/volume ratios. These can have a temporary role or become part of the final product when developed from edible sources [74].

Cells grown in the 2D environment inside or on the surface of the MCs can provide a smooth transition from flasks and bioreactors to finalized 3D tissue outcomes [74]. Different types of marine polymers could be used for cell-cultivated seafood, including hydrogels from algal sources, chitosan extracted from marine exoskeletons, and gelatin from underutilized species such as jellyfish, fish skin, and seafood byproducts, which can also provide specific colors and flavors. In addition, extracellular matrix proteins and lipids can be integrated into the process via scaffolds and can have a significant role in the sensory and textural properties of fish meat. A recent study illustrated that some of the established lipid structure approaches, such as oleogels, could be integrated with cells cultured on microcarriers to form 3D structures simulating meat products [73]. These approaches of combining structured fats with fibrous tissue scaffolds could enable the development of muscle-like fish products; however, there have been few studies reported in the literature to date. Cellular aggregates as self-scaffolding outcomes can also be pursued as a robust option for increasing biomass.

Scale-Up

Scaling up using bioreactors for the 3D cell production environment is a major bottleneck for the cell-cultivated meat industry. Most approaches being pursued are based on variations with stirred tank bioreactors derived from pharmaceutical industry designs, with a focus on cost reductions via simplified designs or those requiring lower energy impacts. These systems apply to cultivated meat and seafood alike. Other approaches generally being pursued in the field include hollow fiber-based bioreactors. In all cases, the costs of scaling are related to media, microcarriers, clean rooms, bioreactor hardware, and labor.

Innovative approaches will be required to reduce the cost of scaling up. For example, there are many unutilized nutrients and growth factors, which could

be recovered and returned to the bioreactor after removing cell metabolites. This could be achieved using different approaches, such as growing plants on the spent media to generate additional biomass for use in the production process, utilizing microbial communities for metabolic support to reduce inhibitor byproducts, along with more traditional selective membranes to isolate, recover, and re-use key growth factors.

Reductions in ammonia can be pursued using microorganisms and chemicals, which can help sustain cultures with reduced media changes or specific nutrient feeding. Glutamine substitutes, including α-ketoglutarate (αKG), glutamate (Glt), and pyruvate (Pyr) had a positive impact on cell proliferation and differentiation by reducing the rate of ammonia production [75]. For instance, proliferation media containing αKG improved primary bovine fibro-adipogenic progenitor cell proliferation, while significantly reducing ammonia production rate due to the antioxidative and ammonia-scavenging properties of αKG [75].

Food safety

In the US, both the Food and Drug Administration (US FDA) and the United States Department of Agriculture (USDA) have established a joint agreement to address cell-cultivated meat and seafood safety and regulations. The FDA oversees cell collection, cell banks, cell growth, and differentiation for all the seafood organisms, while the USDA/Food Safety and Inspection Service (FSIS) evaluates the products after harvest onwards for catfish. Codex Alimentarius also recently initiated programs on developing Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practices (GMP) for cell-cultivated meat and seafood.

Complementary to regulatory organizations, the Food and Agriculture Organization (FAO), and the World Health Organization (WHO) developed the first comprehensive food safety document that covers cell-cultivated seafood [2]. This document outlines the food safety risks, including zoonotic risks from cell lines and the production environment, biological contamination risks from initial cell sources to production, and risks from unwanted residues and novel inputs during production and processing of cell-cultivated meat products.

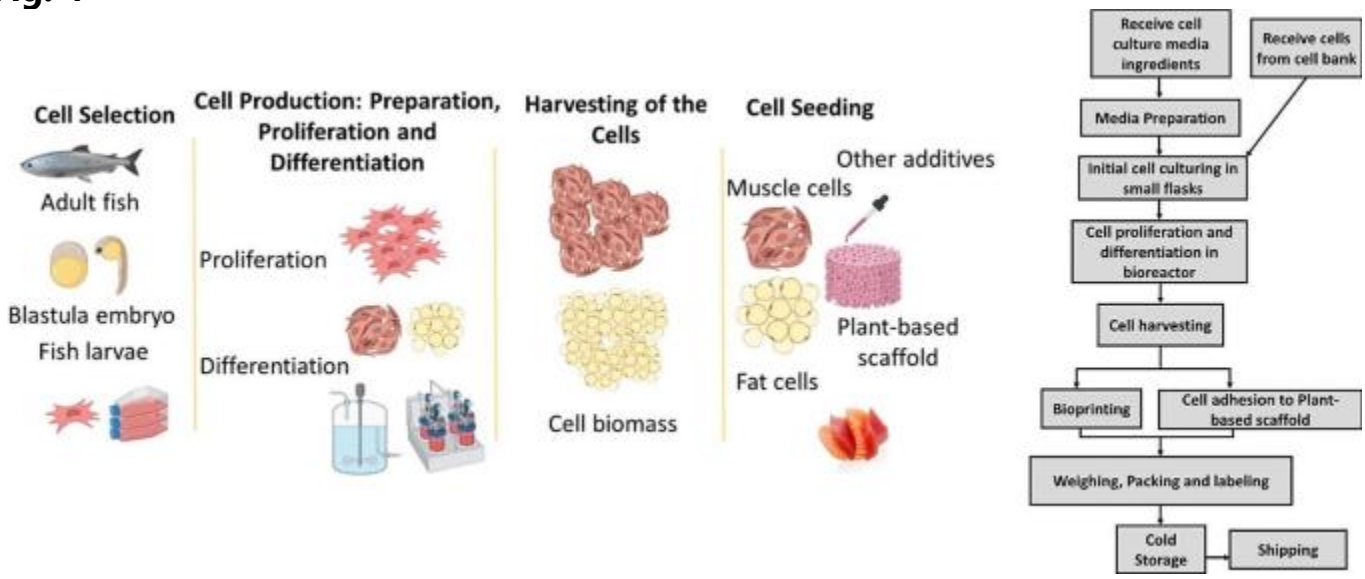
These risk factors are combined with a food safety plan to address the challenges and regulatory requirements of both the FDA and the USDA along each step of cell-cultivated seafood production (Fig. 4). Critical Control Points are biological, chemical, allergen, and physical issues that need to be addressed for developing preventive controls.

In the cell culture environment, bacteria can rapidly outgrow the animal cells, with additional hazards from other organisms, including viruses, prions, fungi, protozoa, and parasites.

Escherichiacoli, *Listeriamonocytogenes*, *Salmonella* spp., *Aeromonas hydrophyla*, *Vibrio spp.*, and *Mycoplasma spp.* are some of the most common bacterial contaminants in foods.

Chemicals may be added intentionally or unintentionally to the production process and can pose food safety risks. These chemicals include antibiotics, drugs, sanitizers, cryoprotective agents, leachable chemicals (including plasticizers), surfactants, and anti-foaming agents. The leachable chemicals can originate from sensors and piping in the production process. There are approved chemicals listed by the FDA that can be used for cell culture, but for new production processes, these potential contaminants will need to be tracked. Physical hazards include objects, debris, plastics, and microplastics.

Fig. 4



(**A**) Cell-cultivated seafood production steps; (**B**) Cell-cultivated seafood production critical control points

A major issue with seafood is the allergens, with different types of proteins and allergens in fish and shellfish. For example, the major allergens in fish are parvalbumins, while in shellfish, tropomyosin, arginine kinase, and myosin light chain are the main allergens [76]. Cellular aquaculture has the potential to reduce allergenicity in seafood by selectively growing specific cell types (e.g., myogenic and adipogenic cells) to avoid allergenic components. This can also be achieved through genetic modifications, such as using RNA Interference (RNAi) techniques to knock out causative genes [77]. Additionally, the incorporation of food-grade additives like creatine or ethylenediamine tetra-acetic acid (EDTA) into the cell culture media may offer a route to address allergen-related issues by modulating the expression of parvalbumin, thereby reducing allergenicity [78].

Regulations

Cell-cultivated seafood industries need to comply with the preventive controls rules established by the Food Safety Modernization Act (FSMA). According to the FDA, "Generally, domestic and foreign food facilities that are required to register with the Sect. 415 of the Food, Drug, & Cosmetic Act must comply with the requirements for risk-based preventive controls mandated by the FDA FSMA as well as the modernized Current Good Manufacturing Practices (CGMPs) of this rule (unless an exemption applies)".

Traditionally, the conventional seafood industry is regulated by the FDA, except for catfish (*Siluridae*), which, along with meat products, is regulated by the USDA [79]. Cell-cultivated seafood production is considered a novel or alternative food production system. Thus, labeling is also an important part of the regulations for food products. Developing a common terminology to increase transparency is required for clean labeling.

There was a comprehensive study for seafood products indicating that two "common or usual names," "Cell-cultivated Seafood" and "Cell-Cultured Seafood," met regulatory criteria [80]. By displaying these two phrases on packages of frozen Atlantic Salmon, both "Cell-cultivated" (60.1%) and "Cell-

Cultured" (58.9%) enabled participants to differentiate cell-cultivated seafood from "Farm-Raised" and "Wild-Caught" fish [80].

There is a need to develop reliable test kits and rapid detection sensors to validate the safety of cell-cultivated seafood products. Testing methods are essential for assessing allergenicity in seafood products, including those produced through cellular aquaculture. These methods need to encompass not only the cultured cells themselves but also the biomaterial scaffolds employed in the process [81, 82]. *In silico* assessments can determine sequence homologies and identify structural similarities of newly expressed proteins to existing allergenic examples [83] while other testing methods approved by the EFSA and the FDA for allergenicity verification include the pepsin resistance test and immunochemical cross-reactivity testing with Immunoglobulin E (IgE) from the serum of allergic individuals [84, 85].

Traceability of cell-cultivated seafood will also be a major topic, as is the case with conventional meat products. The conventional seafood industry is highly fragmented, with very little connection from the point of harvest to the point of consumption. In contrast, cell-cultivated seafood could be easily traced back to the source of production.

Socioeconomics

One concern with cell-cultivated seafood is that, in the future, by developing this novel food production system, the declining need for animals, including fish and crustacea, could negatively impact the fishing industry and the associated communities [86]. However, cell-cultivated seafood is strategically positioned to complement traditional methods like wild-caught species and aquaculture farming, to support the sustainability of these communities well into the future. Moreover, the capacity to harvest and culture cells from unconventional seafood sources provides new possibilities for these communities, simultaneously enriching food choices available to consumers. Figure 5 summarizes some of the benefits and challenges/concerns associated with the cell-cultivated seafood industry.

Fig. 5

Potential societal impacts of cell-cultivated seafood [86,87,88]

Industrial scale

Businesses involved in cell-cultivated meat, including seafood, have been gaining significant importance across the globe, reflected in investments of about $2.8 billion since 2016 among 156 companies dedicated to cell-cultivated meat and seafood production [89]. Cell-cultivated seafood is an important niche within the cell-cultivated protein sector [90] with industrial investment of $896 million for cell-cultivated meat and seafood [91], with many startups and established companies pursuing cell-cultivated seafood in 2022. This includes companies in the US, Singapore, Europe, Canada, South Africa; Israel, South Korea, Hong Kong, and India. The majority of companies are focused on business-to-consumer (B2C) and business-to-business (B2B), with fewer companies in the B2B business model space [89]. Supply chain issues of cell-cultivated seafood will also need to be addressed as the market expands. The market potential for cell-cultivated seafood remains unknown at the early stages, with price being one of the determinants. Costs are expected to decrease with cheaper ingredients and with scaling, but this has to be demonstrated in the coming years [92].

Transformative potential of cell-cultivated seafood

Cell-cultivated seafood as a technology offers a potentially transformative impact for foods of the future. This is based on the scientific tools now available, coupled with the features of the technology itself. For example, the potential to directly alter cell composition (e.g., fatty acid profiles) to provide healthier seafood products is compelling (see omega-3 example below). This impact can be further enhanced pending the acceptability of GM-based approaches, where seafood cells can be bioengineered to provide even further nutritional and perhaps even therapeutic benefits. Food safety can also be greatly enhanced, as shelf life, microbial community, tracking, and overall freshness can potentially be improved, along with a major reduction in antibiotic use [93, 94].

All of these potential benefits remain to be demonstrated as the field moves forward, but the underlying science to achieve such goals is already in place. In addition, improved food security, food access, novel foods, and many other future outcomes can be anticipated.

Nutrition - Omega-3s and other inputs - Although fish are recognized as one of the best sources of nutritionally important long-chain omega-3 fatty acids, the source of these compounds is the marine algae, bacteria, and protists. Fish consume these organisms either directly or indirectly via other fish or zooplankton, thereby bioaccumulating omega-3 fats in their tissues [95]. The fact that animal cells—including those of fish and aquatic invertebrates—are incapable of synthesizing omega-3 fats *de novo* means that producers of cell-cultivated seafood will need to acquire appropriate sources of omega-3 fatty acids as ingredients. These sources could include farming of microalgae [96], precision fermentation [97, 98], plant molecular farming [99, 100], or cell-free systems [101]. However, this latter strategy has not yet been explored for omega-3 production to our knowledge, and the former three strategies will still require substantial effort before they can be scaled to the levels that may be required to support the cell-cultivated seafood industry.

Cellular engineering approaches could also provide an opportunity to engineer fish cells to synthesize long-chain omega-3 fatty acids. Codon-

optimized transgene expression of omega-3 desaturase gene (*fat1*) of *C. elegans* in a fish cell culture and zebrafish model enhanced the conversion of n-6 PUFA to n-3 PUFA [102]. This study also illustrated that combined transgene expression of *fat-1* and *fat-2* enhanced the synthesis of n-3 PUFA [102].

In addition, cellular engineering may provide a potential solution to enhance the accumulation and stability of omega-3 fats. These approaches may include the use of exogenous reactive oxygen scavengers in the media to promote cell proliferation and suppress oxidation processes [101], as well as genetic modifications to over-express antioxidant genes, such as superoxide dismutase (SOD). Furthermore, cellular engineering approaches also enable the design of media compositions to promote the synthesis of omega-3 fats [103].

Other compounds with important impacts on nutrition and organoleptic properties of seafood are also ultimately derived from the diets of aquatic animals. This includes the carotenoid astaxanthin, which is responsible for the color of salmon and shrimp, as well as for protecting membrane lipids from oxidation [104]. As is the case with omega-3 fats, astaxanthin, and other compounds that are diet-derived in conventional seafood will need to either be sourced as ingredients for addition to cell-cultivated seafood or synthesized by engineered cells.

Notably, the U.S. government recently acknowledged the need to "bolster research into alternative feed ingredients for livestock and aquaculture, including plants, algae, or seaweeds, that can enhance or replace feed ingredients" [105]. Marine-derived feed ingredients such as omega-3s and astaxanthin may be a shared need across both conventional and alternative protein production platforms.

Roadmap and conclusions

Cell-cultivated seafood is in its infancy. There is growing research among academic labs and a growing corporate effort, mainly among startup companies worldwide, to tackle the increasing consumer demand for seafood. In these early stages, the focus is on cell sources, media optimization, and

scaffolding, while with time these efforts will mature into scaling production for impact. With scale, pricing will be reduced and availability will increase. The vision is that this emerging approach to cell-cultivated seafoods will offer safer and healthier alternatives for consumers, while enhancing environmental sustainability goals (Fig. 6).

Fig. 6

Roadmap for the development of the cell-cultivated seafood industry. High-level research and commercial priorities are indicated based on the approximate timing with which they are likely to be high priorities for the

field. MC: microcarrier, STB: stirred-tank bioreactor, HFB: hollow fiber bioreactor

For this growing industry to reach its potential, government support for research and commercialization efforts will be essential. A report by the UK Foreign, Commonwealth & Development Office and the ClimateWorks Foundation estimated that annual global public spending on R&D and commercialization—including that of plant-based proteins, precision fermentation, insects, and cell-cultivated meat—would need to increase to a total of US $10.1 billion to unlock the full benefits of alternative proteins [106].

Whereas terrestrial cell-cultivated meat benefits from a strong foundation of biomedical tissue engineering research and a fairly detailed understanding of mammalian and avian cell biology generally, this is less true for cell-cultivated seafood. Therefore, basic research aimed at understanding piscine and invertebrate cell types, differentiation processes, and metabolic requirements is still needed. Public funding of such research will reduce duplication of effort and provide a strong foundation for commercial efforts, thereby benefiting the field as a whole, everyday consumers, and the planet. Universal in this evolution to grow cell-cultivated seafood as a major option for alternative food for consumers around the world, safety, flavor, and texture are paramount.

Thus, regulations and methods to properly assess these new foods and to provide tracking will be a foundational need. In total, the potential for this emerging field to transform the seafood that we consume, while providing major benefits to sustainability, quality, and food safety, is expected to continue to drive the growth of this field. Both fishing and aquaculture already face major environmental challenges, and cell-cultivated seafood offers a new approach to address these issues, while also expanding our palates in ways never before possible [107, 108]. The future is exciting, but the path will need to be built upon a strong scientific foundation linked to consumer willingness to try these new foods and eventually to embrace them.

Eating fish is linked to better sleep and a higher I.Q. for kids

Kids who eat fish at least once a week sleep better and have IQ scores that are 4 points higher, on average, than those who eat fish less frequently or not at all, a new study shows.

Previous studies showed a relationship between omega-3s, the fatty acids in many types of fish, and improved intelligence, as well as omega-3s and better sleep. But researchers hadn't connected all three before.

The findings reveal sleep as a possible mediating pathway, the potential missing link between fish and intelligence.

"This area of research is not well-developed. It's emerging," says Jianghong Liu, associate professor of nursing and public health at the University of

Pennsylvania and lead author of the study, which appears in Scientific Reports. “Here we look at omega-3s coming from our food instead of from supplements.”

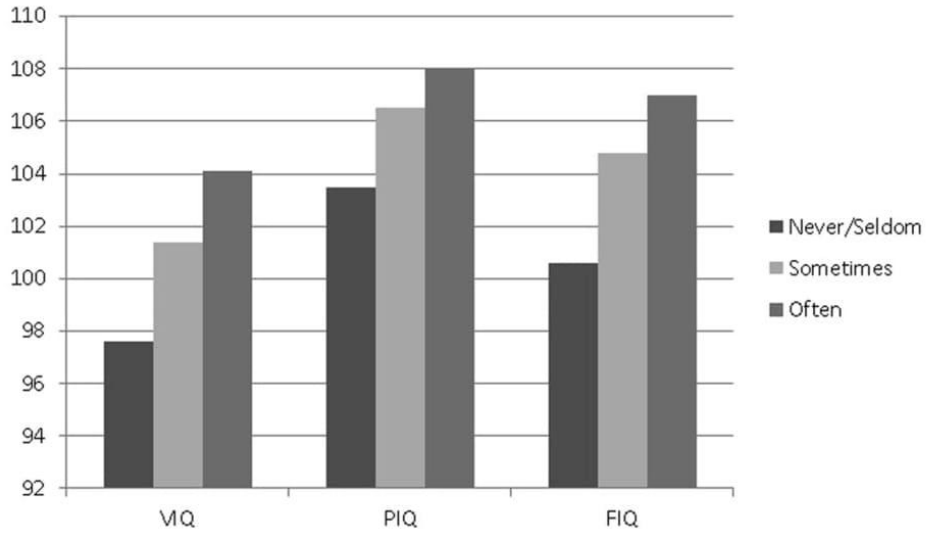
For the work, a cohort of 541 9- to 11-year-olds in China, 54 percent boys and 46 percent girls, completed a questionnaire about how often they ate fish in the past month, with options ranging from “never” to “at least once per week.”

Children also took the Chinese version of an IQ test called the Wechsler Intelligence Scale for Children-Revised, which examines verbal and non-verbal skills such as vocabulary and coding.

Their parents then answered questions about sleep quality using the standardized Children's Sleep Habits Questionnaire, which included topics such as sleep duration and frequency of night waking or daytime sleepiness. Finally, the researchers controlled for demographic information, including parental education, occupation, and marital status, as well as the number of children in the home.

The findings show that children who reported eating fish weekly scored 4.8 points higher on the IQ exams than those who said they “seldom” or “never” consumed fish. Those whose meals sometimes included fish scored 3.3 points higher. Further, increased fish consumption was linked to fewer disturbances of sleep, which the researchers say indicates better overall sleep quality.

From: The mediating role of sleep in the fish consumption – cognitive functioning relationship: a cohort study



Means of IQ measures by fish consumption groups.

“Lack of sleep is associated with antisocial behavior; poor cognition is associated with antisocial behavior,” says Adrian Raine, a professor with appointments in the School of Arts and Sciences and the Perelman School of Medicine. “We have found that omega-3 supplements reduce antisocial behavior, so it’s not too surprising that fish is behind this.”

Jennifer Pinto-Martin, professor of nursing and epidemiology and also executive director of the Center for Public Health Initiatives, sees strong potential for the implications of this research.

“It adds to the growing body of evidence showing that fish consumption has really positive health benefits and should be something more heavily advertised and promoted,” she says. “Children should be introduced to it early on.” That could be as young as 10 months, as long as the fish has no bones and has been finely chopped, but it should start by around age 2.

“Introducing the taste early makes it more palatable,” Pinto-Martin says. “It really has to be a concerted effort, especially in a culture where fish is not as commonly served or smelled. Children are sensitive to smell. If they’re not used to it, they may shy away from it.”

Given the young age of the study group, researchers chose not to analyze the details participants reported about the types of fish consumed, though they plan to do so for work on an older cohort in the future. They also want to add to this current observational study to establish, through randomized controlled trials, that eating fish can lead to better sleep, better school performance, and other real-life, practical outcomes.

For the moment, researchers recommend incrementally incorporating additional fish into a diet; consumption even once a week moves a family into the “high” fish-eating group as defined in the study.

“Doing that could be a lot easier than nudging children about going to bed,” Raine says. “If the fish improves sleep, great. If it also improves cognitive performance—like we’ve seen here—even better. It’s a double hit.”

The National Institutes of Health/National Institute of Environmental Health Sciences grants and the Intramural program of the National Institute on Alcohol Abuse and Alcoholism funded the work.