Meeting the Needs of the Cell-Based Meat Industry

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Commercialization of cell-based meat products at economically viable prices will require significant innovations, presenting new challenges and opportunities for industrial biotechnologists.

Cell-based meat (also referred to as clean or cultured meat) is genuine meat cultivated directly from animal stem cells rather than by raising and slaughtering animals (Figure 1). The meat is created through a bioprocess in which stem cells are extracted, isolated, and proliferated in bioreactors at high densities and/or in large volumes. These stem cells are subsequently differentiated, either in the presence or absence of scaffolding materials, into the principal cellular components of meat, including skeletal muscle, adipocytes, and fibroblasts of the connective tissues. The final product mirrors the structure, composition, and nutritional value of conventionally derived meat.

Advances in regenerative medicine and bioprocess engineering have made the creation of palatable prototypes relatively straightforward. However, scaling up the process while lowering costs will require innovations in cell line development, cell-culture-medium optimization, bioreactor and bioprocess engineering, and scaffold biomaterials.

A growing problem

The United Nations estimates that by the year 2050 there will be 9.7 billion humans on Earth. As this number grows, the socioeconomic status of residents in developing countries will continue to increase, and global demand for meat is expected to double *(1)*. This appetite for meat from industrialized animal agriculture is not without consequence.

Animal agriculture accounts for 14.5% of global greenhouse gas emissions *(2)* and is projected to account for 81% of the remaining carbon budget under the Paris Agreement by 2050 if current rates of production continue *(3)*. While 77% of habitable land on Earth is used to raise and feed livestock, this land use accounts for only 17% of the global caloric supply *(4)*. Industrial animal agriculture is the leading cause of global deforestation and biodiversity loss *(5)*, and it is a major contributor to foodborne illness and zoonotic disease outbreaks *(6)*. The volumes of antibiotics used to produce livestock and farmed fish is at least equivalent to that used in humans, and antibiotic use is expected to rise, making industrial animal agriculture a significant contributor to antibiotic resistance *(7)*.

The public awakening to the urgency of climate change and the negative externalities associated with industrial

▲ **Figure 1.** This meatball is formed from cell-based meat that was grown in a bioreactor from bovine stem cells, eliminating the need for livestock and the associated ethical and environmental challenges. Photo courtesy of Memphis Meats.

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animal agriculture, including animal welfare, has made consumers more accepting of alternative meat products, such as plant-based and cell-based meat *(8)*.

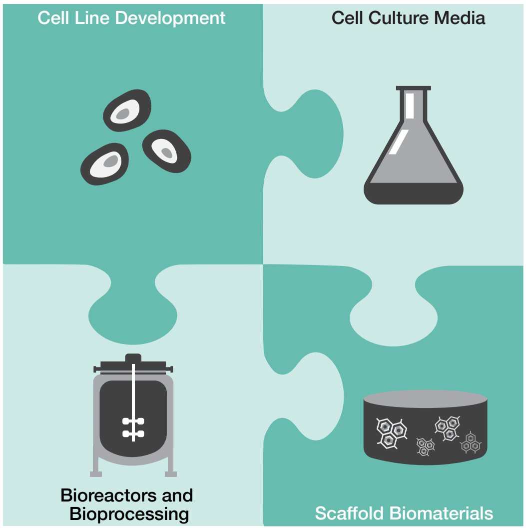
A potential solution

Growing crops to feed animals to produce meat is a vastly inefficient process, as most calories are expended for metabolism rather than creating edible meat. In 2013, Mark Post revealed the first cell-based hamburger, demonstrating that the animal could be cut out of the equation altogether. Since then, more than three dozen cell-based meat companies have formed across the world, aimed at dramatically reducing negative externalities of meat production while taking a bite out of the more than $1 trillion global market.

Preliminary projections estimate large gains in land use and energy efficiency and reductions in eutrophication (*i.e.,* nutrient runoff from fertilizers and manure that cause algal blooms and water dead zones) *(9)*, as well as curtailment of livestock-related biodiversity loss and zoonotic disease. At scale, preventive controls and monitoring methods adapted from existing biopharmaceutical bioprocesses enable antibiotic-free cultivation, lowering global antibiotic use while simultaneously reducing the incidence of foodborne illness. These benefits make cell-based meat a potential solution to many pressing problems.

Critical technology areas

To commercialize cell-based meat, four critical technology areas require further innovation: cell line development, cell culture media, bioreactors and bioprocessing, and scaffold biomaterials (Figure 2) *(10)*.



▲ **Figure 2.** To reach price parity with conventionally derived meat, engineering of cell lines and bioreactors is needed alongside smart selection of raw materials for cell culture media and scaffolding.

Cell line development

As starting material for cell-based meat, cells that can self-renew and differentiate into the cellular components of meat are isolated and selected. Companies in the cell-based meat space work with embryonic, induced pluripotent, mesenchymal, and adult stem cells such as myosatellite cells. The starting cell type ultimately influences many of the downstream variables of the bioprocess, such as timeline and differentiation strategy. Cell selection should be weighed alongside cost models and design requirements for the intended products.

Considerable work has been done using these cell types from bovine and porcine species, but substantially less work has been performed on the range of other species humans consume, especially sea creatures. Publicly available biorepositories of cell lines from commonly consumed species are needed to accelerate research and generate -omics datasets to facilitate development.

A variety of cell line engineering strategies can improve upon or optimize the bioprocess. However, future regulatory standards may dictate the extent to which engineering appears in final products. For example, strategies might include the creation of immortalized cell lines and cells that have high tolerance to shear stress, resistance to toxic metabolite buildup such as ammonia and lactic acid, suitability for suspension growth, and low growth factor concentrations. Engineered biosensors can assist in signaling hypoxic conditions, mechanical stress, or amino acid and glucose starvation *(11)*. Other strategies may be able to remodel metabolic or differentiation pathways, making them more efficient or favorable to low-cost cell-culture-medium ingredients, rather than expensive growth factors.

Researchers may pursue cell lines that inherently exhibit many of these properties, such as insect cell lines that are adaptable to suspension growth, tolerate nutrient starvation, and readily immortalize *in vitro,* or fish cells that can be grown at room temperature *(12, 13)*. Companies and researchers with experience in strain optimization or high-throughput genome editing are needed to support these efforts.

Cell culture media

The cell culture medium is the most important factor in maintaining cells *ex vivo*. Since the 1950s, virtually all basal cell culture media have consisted of variable buffered solutions of glucose, inorganic salts, water-soluble vitamins, and amino acids tailored to specific cell types. To achieve long-term maintenance and proliferation, insulin, transferrin, selenium, lipids, antioxidants, and other growth factors are included, typically in the form of animal sera such as fetal bovine serum (FBS).

FBS has been a mainstay in mammalian cell culture

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because it is rich in growth factors and hormones, which supports a proliferative fetal-like state. However, FBS is not viable for use in cell-based meat because:

- it varies by region and batch
- it is a potential source of contamination
- it is misaligned with animal welfare
- not enough of it is available to supply the industry *(14)*.

While serum-free alternatives exist, they are expensive and often optimized for human cells in clinical settings or cell lines used in production of biologics under current good manufacturing practice (cGMP) guidelines. Estimates suggest that 55–95% of the marginal cost contribution of a cell-based meat product will come from the medium. Thus, the cell-based meat industry will likely require optimized serum-free formulations for a variety of cell types, at price points below $1.00 per liter to become economically feasible at industrial scales *(15)*.

Several strategies could help achieve this goal. For example, protein-rich hydrolysates from plants, such as soy, wheat, pea, or organisms such as yeast and cyanobacteria, can support a proliferative environment for cells at low cost *(16)*. Machine learning or differential evolution algorithms could be used in tandem with *in silico* modeling or high-throughput microfluidic systems to accelerate the pace of formulation discovery *(17)*.

Production of commonly used recombinant proteins, such as insulin, transferrin, FGF2, TGFβ, and platelet-derived growth factor (PDGF), must be scaled to match production costs of food industry enzymes such as pectinase and cellulase, which can be purchased for less than $5.00 per gram. This may require additional host or protein engineering, as certain growth factors, such as TGFβ, are typically produced in mammalian expression systems rather than microbial host platforms. The growth factors themselves may also be engineered to create synthetic proteins with multiple bioactive domains or more-stable isoforms.

Recent demonstrations focusing on the optimization of growth factor production suggest that stem cell medium costs can be reduced by 97% or more *(18)*. Lower purification demands for food-grade production of basal and recombinant components may reduce costs further, but may also require new, nonpharmaceutical-grade manufacturing facilities. It is unclear whether regulations or the need for reproducibility will require chemically defined medium formulations; the answer may dictate the exclusion of medium constituents such as hydrolysates, which are chemically undefined.

Additional methods to reduce costs include the development of small molecules that can mimic the bioactivity of more-expensive growth factors. However, the safety profile of any residuals within a final product should be considered for this approach. Water and nonmetabolized medium components could be recycled using size-exclusion dialysis filters to reduce costs while simultaneously removing waste *(19)*. Efforts by the biopharma industry to move toward perfusion culture and continuous bioprocessing have driven the development of continuous monitoring systems and adaptive control with concentrated feeds, which could also help lower the cost of cell-based meats.

None of these strategies are technologically infeasible or require large scientific leaps. Rather, the demand being established by the ultra-large-volume cell-based meat industry is driving the effort to rethink the composition of cell culture media. New business opportunities abound for those equipped to scale recombinant protein production and rapidly iterate media formulations.

Bioreactors and bioprocessing

In order to scale beyond taste tests toward market readiness, standard 2D culture or miniaturized stirred flasks must be replaced by bioreactors capable of supporting high-density and/or large-volume cell cultures. Production of biologics using suspension-adapted cells in stirred-tank reactors has reached volumes of 20 m^3. But, the production of therapeutic off-the-shelf mesenchymal stem cells typically uses volumes less than 0.25 m^3, as these cells must be cultured on microcarriers or another solid surface to avoid a form of programmed cell death known as anoikis *(20)*. Cells used in cell-based meat are also anchorage-dependent and face similar challenges. Thus, significant developments are needed to scale cell-based meat to affordably and reproducibly produce batches upward of 10^{12} to 10^{15} cells.

Scaling up can require large capital expenditures and time. To increase scaling efficiency, miniaturized bioreactors or microfluidics can produce predictive models of process parameters. Once the process works at larger scales, development of real-time, online sensor systems can help enable continuous and/or perfusion bioprocessing methods that save money. *In silico* modeling of nutrient utilization and the buildup of inhibitory or stimulatory paracrine factors and/or toxic waste can inform feeding strategies, timelines, and perfusion rates *(21)*. The implementation of automation from the ground up, as opposed to retroactively replacing manual steps, can unlock additional cost savings. Dynamic cost-of-goods models can help identify bottlenecks that can be prioritized for automation or future research and development (R&D) efforts as the industry matures.

Proliferation of cells in a semi-continuous or continuous process can minimize processing times or increase the productivity of seed train processes. In a seed train process, cells are grown and used to inoculate sequentially larger, higher-volume vessels, capturing the greatest efficiencies at later cell doublings. For example, productivity can be increased by using a percentage of cells from the highest-

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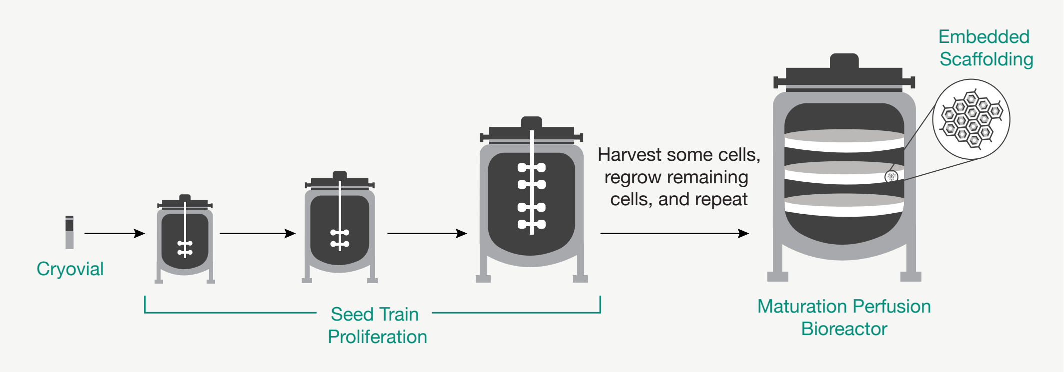

Figure 3. Seed train processes may be semi-continuous or continuous. In this process, cells are grown and used to inoculate sequentially larger vessels in higher volumes. Productivity can be increased by using a percentage of cells from the highest-volume vessel in the proliferation stage to directly inoculate a final, large-volume maturation perfusion bioreactor.

volume vessel in the proliferation stage to directly inoculate a final, large-volume maturation bioreactor (Figure 3).

Perfusion bioreactors, such as hollow-fiber bioreactors, can achieve higher cell densities in lower volumes and operate continually over months, making them an attractive conduit between proliferation seed-train stages. Additionally, larger-volume reactors can be directly inoculated using high-density cryobanking at greater than 10^8 cells/mL, lowering the time to achieve desired cell densities or numbers in seed trains *(22)*.

Innovations such as cell-laden core-shell hydrogels can achieve remarkably high densities of 5×10^8 cells/mL, permitting cellular proliferation in 3D microenvironments shielded from shear stress *(23)*. Creative approaches that entail thinking beyond what has worked for cell therapy may prove to be a valuable strategy for those moving into the cell-based meat space.

While cell therapy and cell-based meat both share the cell itself as the end product, the final stages of cell-based meat — differentiation and harvesting — will likely look quite different. Although unstructured meat products could themselves be composed of pressed cells, cells as additives, or even cells on edible microcarriers, structured products will require the use of a scaffold. Computational fluid dynamics (CFD) models are needed to understand how fluids in a perfusion bioreactor with embedded scaffolding behave. Online sensors can be used to adjust flowrates as the scaffold becomes cell-laden to protect the cells and scaffold itself from fluctuating shear forces. Bioreactor and bioprocess engineers are needed to create new bioreactor models that can support this culture strategy while integrating straightforward harvesting and sterilization processes.

Scaffolding biomaterials

A scaffold for cell-based meat ideally permits cells to attach and differentiate in a specified manner that mimics the 3D cytoarchitecture of an intended meat product. The cytoarchitecture must allow for continuous perfusion of media, analogous to the vascularization of real tissue. In tissue engineering, considerations of the porosity of the scaffold, mechanical properties, and biocompatibility are paramount; in creating cell-based meat, the use of cost-effective edible or biodegradable materials is just as important. However, cell-based meat does not require the same microscale precision as functional tissue. It merely needs to represent tissue structure sufficiently to replicate the appropriate texture and mouthfeel.

Further exploration of plant- or fungal-derived polymers as scaffolds is needed. These organisms may be engineered to express key cell adhesion domains used by vertebrates to boost biocompatibility *(24)*. Alternatively, a polymer-based scaffold could be enzymatically modified or embedded with growth factors to temper the dynamic cellular behavior following seeding. Chemical modifications can create a tunable scaffold that is responsive to simple external stimuli such as light or temperature *(25)*. These or other forward-thinking strategies related to preferred materials and how they may be sourced via existing or new supply chains can help encourage the development of cell-based meat.

Methods pioneered by tissue engineers can be adopted for the assembly of cell-based meat scaffolding but will need to be expanded upon. For example, extrusion and stereolithographic bioprinting are promising candidates, but these processes must be able to be run economically at large scales in parallel. Use of electrospinning and decellularization techniques can be informative from an R&D perspective, but may be difficult to implement at scale. Databases with information on plant, fungal, and microbial biopolymer mechanical properties, biocompatibility, anisotropy, viscosity, and other parameters can inform the selection of the most promising candidate methods and materials.

Looking forward

Cell-based meat is a nascent but rapidly growing field that may significantly benefit human, animal, and planetary health. It is a highly interdisciplinary field that presents fascinating scientific challenges, as well as potentially lucrative new market entry points. Challenges for cell-based meat are

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not problems to be faced by the industry alone, but problems to be tackled in collaboration with other fields, such as cell therapy, regenerative medicine, and fermentation products, as the solutions will have a rippling effect.

To have the greatest impact on solving the world's toughest challenges, scientists, engineers, and biotechnologists should consider cell-based meat as an opportunity to apply their skillsets. An influx of talented scientists from across these fields will be needed to further drive the success of the industry. **CEP**

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